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THE LYMPHOCYTE CONTENT OF RABBIT BONE MARROW

By J. M. YOFFEY* and JOSEPHINE PARNELL

From the Department of Anatomy, the University, Bristol

This work has been undertaken with the primary object of studying quantitatively the lymphocyte content of bone marrow. During the progress of the work other data have been obtained, which are briefly referred to here, but which it is hoped to present more fully later on.

The literature concerning lymphocyte production has been previously reviewed (Drinker & Yoffey, 1940). In addition, mention must be made of the work of Sanders, Florey & Barnes (1940), Kindred (1940, 1942), and Erf (1940). The essential facts are that lymphocytes are daily entering the blood stream in large numbers, and disappearing from it in equally large numbers, so that the level of lymphocytes circulating in the blood remains approximately constant. How and where so many lymphocytes leave the blood is still an unsolved problem. They do not appear to be destroyed while in the blood itself (Bunting & Huston, 1921). There is no extensive circulation of lymphocytes between blood and lymph (Yoffey & Drinker, 1939). They are not excreted into the lumen of the alimentary canal. The experiments of Yoffey (1942) on this point were not conclusive, but those of Erf (1940) on rabbits appear to be so. Erf's experiments show that lymphocytes can rapidly disappear from the blood even in the absence of the spleen and the major part of the alimentary canal. There thus remains for consideration the only other destination which has so far been suggested for the blood lymphocytes, namely, the bone marrow.

The technique of sternal puncture for the examination of living bone marrow has come into widespread use since its introduction by Arinkin (1929), and has yielded valuable information concerning the qualitative composition of the marrow. But in sternal puncture there is aspirated a mixture of marrow and blood, in varying proportions, which is useless for obtaining absolute marrow counts. The literature of bone marrow deals mainly with differential counts, and contains only scattered references to absolute values.

MATERIAL AND TECHNIQUE

The basic principle of the technique employed was to place a weighed quantity of bone marrow in a known weight of plasma, and then attempt to break up the marrow so that its cells would become freely suspended in the plasma. The cell suspension thus obtained is treated exactly like blood for the purpose of obtaining absolute and differential counts.

The first method employed (Exps. 1–10) was to weigh a small watch-glass, put in some plasma, weigh again, drop in the bone marrow, and weigh a third time. The marrow is then cut up into fragments with two fine knives and the suspension transferred with a pipette to a small corked tube, which is shaken vigorously by hand for about 2 min. This method is open to the objection that some evaporation occurs while the marrow is being teased. It also suffers from the more serious disadvantage that the plasma, although heparinized, frequently clots and renders the experiment valueless. Several experiments performed with the watch-glass technique had to be discarded.

The second and better method (Exps. 11–12) was to drop the marrow into a small, clean, tightly corked glass tube (length 5 cm., internal diameter 1·0 cm.) about half filled with plasma. As with the watch-glass method three weighings are required—with the tube empty, after the addition of plasma, and again after adding the bone marrow. The corked tube, containing known weights of plasma and bone marrow, is now placed in a shaker and shaken for 5 min., making a 10 in. excursion 400 times per minute. At the end of this time most of the bone marrow cells will be found free in the plasma. If counts and smears are made immediately after the shaking is complete, clotting is not usually a source of trouble.

Adult rabbits were used throughout, presumably healthy, but of unknown age. After making a blood count, the animal was anaesthetized by intravenous nembutal, a cannula introduced into one of the carotids, and 9 c.c. of blood collected in a graduated centrifuge tube containing 1 c.c. of a 3 % heparin† solution in normal saline. The animal was then bled to death, to exsanguinate the marrow as far as possible; the heparinized blood previously obtained was centrifuged for half an hour at 3000 r.p.m., and the supernatant plasma then withdrawn.

A rib, usually the fourth or fifth, was then removed, and scraped clean down to the bone. The outer wall of the rib was cut away with fine pointed scissors to expose the marrow. With a fine needle

* Aided by a grant from the Colston Research Fund.

† For the heparin used in these experiments we are greatly indebted to British Drug Houses Ltd.

this is worked loose, and dropped in varying amounts into the plasma-containing tube, which is then weighed and shaken as previously described.

All marrow and blood films were stained with Wright's stain. At the same time, to obtain lymphocytes for purposes of comparison, suspensions were made of cells in the glandula mesenterica magna (the single large lymph node at the root of the mesentery), a piece of which was placed in a watch-glass containing some plasma and teased into small fragments. Incidentally, some of these cell suspensions showed very convincing transition series between reticulum cells and large lymphocytes.

The technique described, though the best we have so far been able to devise, has some obvious defects. In our calculations, we have assumed the specific gravity of bone marrow and plasma to be 1,0, and have taken the weight in grams as equivalent to the volume in c.c. This was done in order to make comparisons with cells in blood and lymph, which are counted in numbers per unit volume. According to Mechanik (1926) the specific gravity of bone marrow is very nearly 1·0, yellow marrow being a little less, red marrow slightly more than 1·0. Costal marrow, being intermediate in cellularity between the fatty marrow of the long bones and the very red marrow of sternum and vertebrae, will therefore have a specific gravity of practically 1·0.

In the differential counts an appreciable number of cells was listed as unclassified, on account of partial damage. Some of the cells may be so fragile as to be altogether destroyed in shaking. This may apply especially to the megakaryocyte, which was never seen in our marrow smears. Granulocytes in the smears are occasionally seen to have been damaged, but not usually disintegrated to the point where they were completely unrecognizable. If there is any considerable destruction of cells, the counts will be on the low side. This effect will be accentuated in those experiments where some of the marrow fragments remain unbroken after the shaking. It is possible that the factor of cell destruction is partly responsible for the discrepancy between our results and those of Kindred (1942), who obtained a considerably higher average count than that recorded here. Kindred's average count in paraffin sections of rib marrow was 1,960,000 nucleated cells per c.mm., which compares with our average figure of 469,900. His work was done on rats, however, and there may be marked species differences in addition to technical differences.

Table 1. *Absolute counts of total nucleated cells and lymphocytes in rabbit marrow, and lymphocytes per cent of nucleated cells*

No. of animal	Weight kg.	Total nucleated cells per c.mm. of marrow	Lymphocytes per c.mm. of marrow	Lymphocytes % of total nucleated cells of marrow
1	1·7	286,000	51,420	18·0
2	2·7	217,000	12,170	5·6
3	1·8	348,000	50,500	14·5
4	1·5	434,000	70,500	16·25
5	2·6	418,000	29,980	7·17
6	2·2	828,000	144,000	17·4
7	2·2	578,000	57,760	10·0
8	1·9	380,000	66,470	17·5
9	2·2	514,000	30,840	6·0
10	2·85	583,000	105,000	18·0
*11	2·95	712,000	90,660	12·75
*12	2·35	341,000	22,580	6·6
Average	2·25	469,900	61,000	12·48

* In Exps. 11 and 12 the animals were given a subcutaneous injection of Pernaemon 2 c.c. (kindly provided by Organon Laboratories Ltd.) 1 and 2 days respectively before the marrow count. Since the counts appeared to be within normal limits, they have been included in the present series.

RESULTS

These are given in Table 1, and the cells we have designated lymphocytes are illustrated in Pl. 1.

DISCUSSION

Cell identification. 'Lymphocytes' in Table 1 include both small and medium lymphocytes, but not large lymphocytes. Most of the cells are small (Pl. 1, figs. 1–15). No large lymphocytes have been recorded in the differential counts. Cells are occasionally seen which might be interpreted as such, but they have not been classified as lymphocytes in the present work, because of possible confusion with myeloblasts and early pro-erythroblasts (Sundberg & Downey, 1942). Though medium lymphocytes (Pl. 1, figs. 16–20) have been described as well as small, the distinction between the two has been an arbitrary one. The name 'medium', though honoured by tradition, is somewhat vague;

it is retained here to emphasize some significant structural features. However, no actual measurements have been made and no size limits have been fixed for the small and medium lymphocytes, since such limits are unreliable unless one can produce films of constant thickness. Generally speaking, cells have been designated medium lymphocytes when they have been appreciably larger than the average small lymphocyte, or when they have possessed a cytoplasmic rim of fair thickness (Pl. 1, figs. 21–25, 29–30). Most of the lymphocytes in rabbit bone marrow are small (Table 2, and Pl. 1, figs. 1–15).

Table 2. *Absolute counts of small and medium lymphocytes per c.mm. marrow*

No. of exp.	Small lymphocytes	Medium lymphocytes
1	45,700	5,720
2	8,700	3,470
3	44,400	6,090
4	51,000	19,500
5	25,800	4,180
7	40,460	17,300
8	61,700	4,770
11	69,300	21,360
12	17,800	4,780
Average	40,540	9,690

The nuclei of the small lymphocytes are regularly and characteristically pachychromatic (Pl. 1, figs. 1–20, 27–28). Cells of similar size and colouring, but with leptochromatic nucleus, have been regarded as micromyeloblasts. The medium lymphocytes, on the other hand (Pl. 1, figs. 21–25, 29–30), at times present a more difficult problem. Even in the lymph nodes, where the nature of the cells is much less open to doubt, a comparison of the nuclear structure of the small and medium lymphocyte shows that in the latter the nucleus tends to be more open in texture with the chromatin strands finer—in other words, leptochromatic. Pl. 1, fig. 23 shows a medium lymphocyte of this type in the bone marrow. Cells of this type, but with the nucleus still more leptochromatic and with slightly increased basophilia, could readily be interpreted as transitional forms on the way to becoming myeloblasts. They have not been included as lymphocytes in this paper.

It has not been thought necessary to make any attempt to discriminate between the small lymphocyte and the 'primitive' cell of Cunningham, Sabin & Doan (1925), since Sabin herself, in later publications (Sabin, Miller *et al.* 1936; Sabin & Miller, 1938), concluded that the morphological criteria for distinguishing primitive cell from small lymphocyte were not satisfactory.

Total lymphocyte content of bone marrow. Taking the figures of Nye (1931) for the total red marrow in the rabbit (i.e. 1·7 % of the body weight), and an average body weight in our experiments of 2·25 kg., the average volume of red marrow is 38·25 c.c. With a lymphocyte count of 61,000 per c.mm., this would mean a total marrow lymphocyte content of 2330×10^6.

Comparison of marrow and thoracic duct lymphocytes. How does this compare with the number of lymphocytes entering the blood via the thoracic duct? Sanders *et al.* (1940, Table IV), in nine rabbits with an average body weight of 2·52 kg., found an average daily lymphocyte output from the thoracic duct of 4000×10^6. Making a slight adjustment for the difference in weight in our present series, this would become 3560×10^6. It seems clear that, while these calculations can be only approximately correct, the figures are of the same order. The total lymphocyte content of bone marrow is such that, compared with the newly formed lymphocytes entering the blood via the thoracic duct, it could be derived from them. This appears to be the more probable since, as has already been pointed out, no other destination can be suggested for the blood lymphocytes, apart from those already disproved.

It is possible, of course, that the marrow itself contributes lymphocytes to the blood, and that the lymphocytes we have been counting are destined to enter the blood stream and are not derived from it. If this were so, the problem of the blood lymphocytes would be more perplexing than ever. The lymphocyte output, as measured by cannulating the thoracic duct, gives in the rabbit a daily replacement factor (D.R.F.) of 5·0 (Sanders *et al.* 1940). Estimates of lymphocyte output based on thoracic duct lymph are almost certainly minimal (Yoffey, 1936), and the true figure for lymphocyte production will therefore be in excess of this, so that in the rabbit the D.R.F. will be 5+. If one assumes that the bone marrow lymphocytes undergo mitosis once in 24 hr., a not unreasonable rate of division, and the marrow population is kept constant by the migration of excess lymphocytes into the blood, there would be on the basis of our figures a total D.R.F. of 8+. We would be faced with the extraordinary situation that lymphocytes are entering the blood in large numbers, remain there for 3 hr. or less, and then disappear without trace.

The suggested multiplication of marrow lymphocytes raises the question of the frequency with which mitoses are found in these cells. The identification even of resting marrow cells presents at times great difficulty; in the case of cells in mitosis, where finer details of nuclear structure become obscured, accurate identification becomes even more difficult unless there is present some distinguishing element in the cytoplasm such as specific granulation or haemoglobin. Ellerman's (1920) method of measuring the mitotic angle may be some help. In the absence of such measurements, and taking into account the difficulties already

mentioned, we would note for what it is worth our impression that mitoses of marrow lymphocytes are few and far between.

Jordan (1939 *a*, *b*) has consistently advocated the view that the lymphocytes are filtered out of the blood stream in the bone marrow, there to form the precursors of all the other blood cells. The data now submitted appear to lend support to the view that lymphocytes leave the blood to enter the bone marrow, though they do not afford any evidence on the fate of the lymphocytes in the marrow.

SUMMARY

A technique is described for obtaining absolute counts of the nucleated cells of the bone marrow.

The costal marrow of the rabbit (average of 12 experiments) had 469,000 nucleated cells per c.mm., of which 61,000 were lymphocytes; 75 % of these were small, 25 % medium.

The total lymphocyte content of rabbit marrow is of the same order as the number of lymphocytes which daily enter the blood through the thoracic duct.

Though the experiments on which this paper is based have been performed at Bristol, the work was begun in the Department of Anatomy, University College, Cardiff. We would like to place on record our gratitude to Prof. C. McLaren West for his kind encouragement while we were working in his Department at Cardiff.

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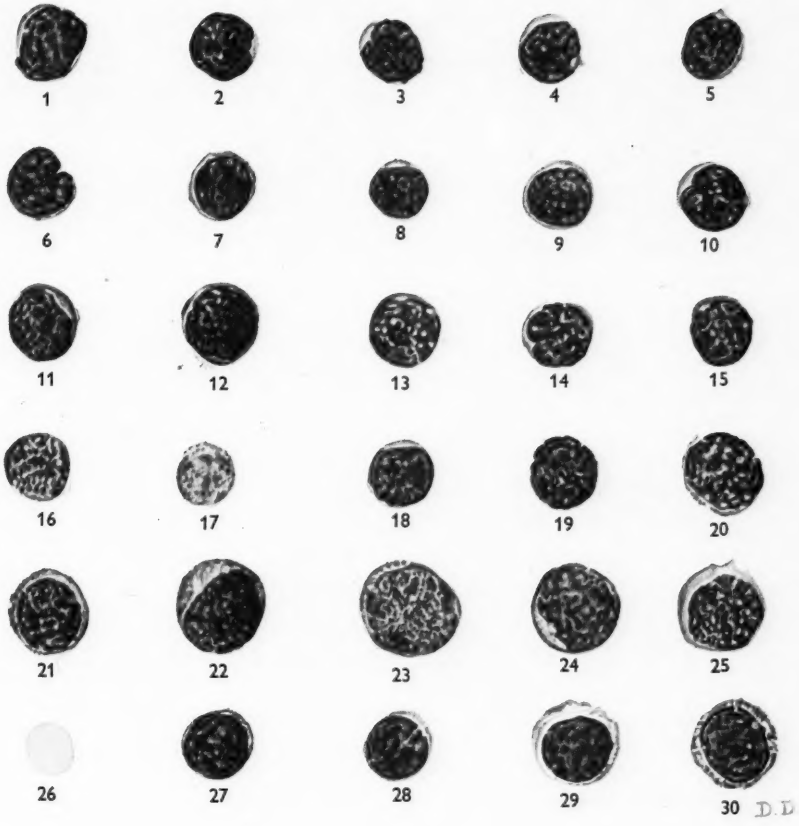
EXPLANATION OF PLATE

Cells from bone marrow, lymph node, and blood. Dry smears stained with Wright's Stain. Drawn with camera lucida, oil immersion ×100, Eyepiece ×12·5.

Figs. 1–15. Small lymphocytes; bone marrow.
Figs. 16–20. Small lymphocytes; glandula mesenterica magna (lymph node).
Figs. 21–25. Medium lymphocytes; bone marrow
Fig. 26. Erythrocyte; blood.
Figs. 27–28. Small lymphocytes; blood.
Figs. 29–30. Medium lymphocytes; blood.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THE CONTRIBUTION OF THE MEDIAL FILLET AND STRIO-HYPOTHALAMIC FIBRES TO THE DORSAL SUPRA-OPTIC DECUSSATION, WITH A NOTE ON THE TERMINATION OF THE LATERAL FILLET

By P. GLEES, *Departments of Anatomy and Physiology, University of Oxford*

Recent papers by Bodian (1940), Tsang (1940), Ranson, Ranson & Ranson (1941), and Magoun & Ranson (1942), have drawn fresh attention to the hypothalamic or supra-optic commissures. These commissures have already been described by the early investigators of neurological anatomy and bear the familiar names, commissure of Gudden or decussatio supra-optica ventralis, commissure of Meynert or decussatio supra-optica dorsalis pars ventralis, and the commissure of Ganser or decussatio supra-optica dorsalis pars dorsalis. As has already been shown by Gudden, they are independent of the optic decussation. The origin and course of the supra-optic commissures (which should be better called decussations, since they do not connect identical structures on two sides), have always been disputed. It had been suggested by earlier workers that the commissure of Meynert is mainly diencephalic in origin and distribution (Weaver, 1937), but that the commissure of Gudden is merely a commissure of the medial geniculate bodies and the corpus parabigeminum (Wallenberg, 1926). Marburg (1927) holds the view that the commissure of Ganser contains fibres from the pallidum, and he terms this connexion the tractus pallido-hypothalamicus cruciatus. Probst (1905) concludes from his investigations, however, that both the commissures of Ganser and Meynert arise from the tegmentum mesencephali. Weaver (1937), from the study of optic tracts which had degenerated for at least two months, came to the conclusion that there is no morphological basis for a division of the supra-optic commissures into a commissure of Meynert, or dorsal supra-optic commissure, and a commissure of Gudden, or ventral supra-optic commissure. Weaver points out the importance of regarding the whole region which includes the commissure of Gudden and Meynert as an inferior hypothalamic decussation and describes it as a complex bundle of myelinated fibres of varying size. According to him the fibres originate in different parts of the diencephalon and merge to a common point of decussation in the anterior hypothalamus below the third ventricle. The greater part of the decussating fibres is found posterior to the optic chiasma. He found no intrachiasmatic component of commissural fibres.

Even the most recent paper of Magoun & Ranson (1942), although making new contributions, still indicates the controversial nature of this subject. In their study of Marchi stained sections of a cat and monkey they describe a dorsal and a ventral supra-optic decussation, and refute the diencephalic origin of these fibres. Both the dorsal and ventral bundles were found to have their origin in the lower brain stem. From this level they ascend to decussate in the inferior hypothalamic region and then descend (at least as far as the fibres of the ventral supra-optic decussations are concerned) to a tectal level in the midbrain.

In our experiments we have found evidence for an origin of the ventral part of Ganser's commissure from the medial fillet and of its dorsal part from the striatum. The origin of this commissure from the medial fillet had previously been suggested by Spitzer & Karplus (1907), who also traced some of the fibres to the pallidum, but, probably due to the unspecific title of their paper, most of the later research workers have neglected their publication. This connexion between medial fillet and pallidum deserves even more interest because of the neurophysiological finding of Dusser de Barenne, McCulloch & Garol (1941) that stimulation of the nuclei of Goll and Burdach has an effect on the electrical activity of the globus pallidus, apparently due to a fibre connexion for which little anatomical evidence was at that time available.

MATERIAL AND METHODS

The cat material used for the study of the connexions of the medial fillet was that employed for a study of the pyramidal system in collaboration with Prof. Liddell (unpublished). Here I wish to thank Prof. Liddell for his generosity in allowing me to use this material. In those experiments where the medial fillet had been injured by lesions involving the medullary pyramids, the Marchi method was employed in order to define the amount and distribution of the ascending and descending degeneration. Some of the material was prepared by the Marchi method of Swank & Davenport, with a slight modification (Glees, 1943), and some by the original procedure given by Swank & Davenport (1934).

The rabbit material here described originated from a study on the connexions of the striatum (Glees, 1944).

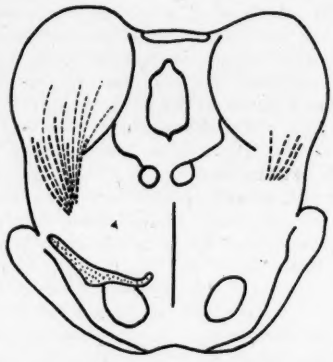
The lesions of the striatum were produced in the caudate nucleus of the cat and rabbit's brain by the insertion of a monopolar electrode consisting of an insulated constantin wire. The current had a strength of 3–5 mA. 12 V. and the flow of the current was maintained for an average of 5 min. In most of the cases the dura remained intact, but for the minute hole through which the needle passed.

EXPERIMENTAL OBSERVATIONS

(*a*) The lesions of the medial fillet of the cats were produced at the level of the lower pons or upper medulla, and the Marchi material of seven cats was examined (cats 1 and 3 of one series and 315, 245, 1910, 1011, 2010 of another series). In cat 3 the left pyramid only was cut at the border of the medulla and pons. Besides the injury to the pyramid the left medial and lateral fillets were also interrupted. Cats 315, 2010, 245, 1910, 1011 and cat 1 had both pyramids cut with a varying amount of injury to the medial fillet of both sides. Large degenerated myelinated fibres emerging from the medial fillet at the level of its termination could be easily followed in all seven cats and were found to contribute to the dorsal supra-optic decussation. The following account is based collectively on the study of all the cat material.

The medial fillet could be traced to terminate mainly within the posterior ventral nucleus of the thalamus. In its course through the midbrain and at the caudal part of the diencephalon numerous other degenerated fibres of medium size could be followed. Some of these cross over by way of the posterior commissure (Pl. 1, fig. 3) dorso-lateral to the fasciculus retroflexus to enter the opposite thalamus and terminate in the most medial part of the posterior ventral nucleus. Besides these, numerous fibres from the terminal part of the medial fillet pass medially and ventrally through the zona incerta to turn further ventro-medially in the direction of the optic chiasma. These fibres cross the midline by passing through Ganser's commissure (Pl. 1, fig. 1), also mingling ventrally with Meynert's commissure, and end partly in the globus pallidus of the opposite side. Still others of these decussating fibres break through the internal capsule of the opposite side, or turn round its lateral border to terminate within the opposite posterior ventral thalamic nucleus.

As regards the lateral fillet, it may be noted incidentally that fibres of this tract could be traced exclusively to the nucleus of the lateral fillet and the posterior colliculus, in agreement with the observations by Woollard & Harpman (1939) and by Barnes, Magoun & Ranson (1943) (see Text-fig. 1). It has always been the classical view on the auditory pathway that the lateral fillet terminates mainly within the medial geniculate body and sends only collaterals to the posterior colliculus, but this conception was found not to be in accordance with the experimental investigations on the lateral fillet by the authors mentioned above. The auditory fibres which reach the medial geniculate body must be considered to be relayed fibres from the superior olive, the nucleus of the lateral fillet, or even a new set of fibres from the posterior colliculus itself. An explanation for this different type of conduction as compared with the lateral geniculate body (which receives direct optic tract fibres) has been offered by Barnes *et al.* (1943). This explanation is based on the assumption that the auditory pathway to the cortex is phylogenetically recently acquired and had to be fitted in an already fixed pattern of nervous pathways. How far the experimental results of the auditory pathways are conclusive for man has still to be investigated; the monkey and mainly the cat possess a large superior olive complex, whereas man has only a rudimentary representation of it.



Text-fig. 1. A camera lucida drawing of a section through the midbrain of cat 3. The stippled area dorsal to the left cortico-spinal tract shows the position of the degenerated fibres of the left medial fillet. The left lateral fillet is seen entering the posterior colliculus. The right posterior colliculus receives some degenerated fibres from the right lateral fillet.

(*b*) Lesions of the striatum in cats 10, 12 and 17 and in five rabbits. The lesions of the caudate nucleus were limited to its head (Text-fig. 2). The resulting degeneration of caudato-fugal fibres could be clearly followed through the putamen or the external capsule to enter the globus pallidus, but the most anteriorly situated fibres pierce the lateral part of the anterior commissure (Pl. 1, fig. 5). The degenerated fibres of small and medium size seem to end for the greater part among the cells of the amygdala, but the thicker fibres sweep medially and caudally and take part in the formation of the dorsal part of Ganser's commissure (Pl. 1, fig. 4 and

Text-fig. 2). After the fibres have crossed the midline, some end in the pars tuberalis, and others in the paraventricular region of the hypothalamus. It is probable that most of the caudato-fugal fibres lose their myelin sheath before their actual termination within the hypothalamus; Marchi preparations, therefore, will not allow a definite statement regarding the actual hypothalamic nuclei in which the fibres end. In some preparations the impression is gained that some of the fibres extend beyond the hypothalamus and are directed to the opposite lentiform nucleus. In the cat most of the degenerated fibres were of medium size, but in the rabbit they were of a coarse calibre.

Text-fig. 2. A camera lucida drawing of the left cerebral hemisphere of cat 12, showing the lesion of the caudate nucleus with some involvement of the internal capsule.

DISCUSSION

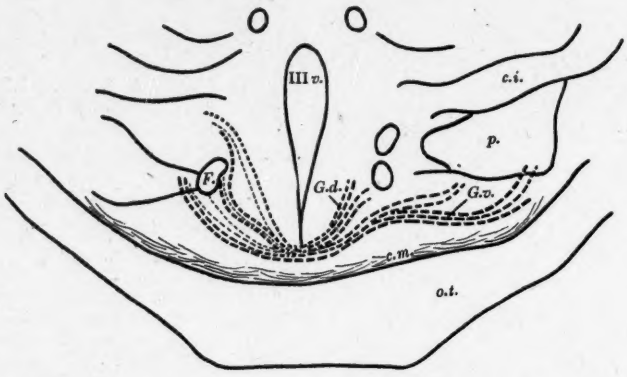
(*a*) *The medial fillet and the pallidum.* By strychninizing the nucleus gracilis or cuneatus Dusser de Barenne and his associates obtained records of strychnine spikes from the globus pallidus. Being unaware of the paper of Spitzer & Karplus (1907) they found almost no anatomical evidence for such a fibre connexion in the literature. As far as this fibre connexion could be demonstrated in our preparations, it is evidently made up of very large myelinated fibres. The coarseness of the fibres (as shown in our material) suggests that they are capable of conveying fast impulses from the proprioceptors of the body to the pallidum via the medial fillet and its nuclei. It seems probable that such an afferent proprioceptive connexion may be of considerable importance in relation to the extra-pyramidal motor pathways originating in the pallidum (see Papez, 1941; and Ranson, Ranson & Ranson, 1941), for it may well provide afferent impulses on the basis of which the pallidum exerts a controlling influence over muscular tone (see Liddell & Philips, 1940). Probst (1905), Spitzer & Karplus (1907) and Magoun & Ranson (1942) added experimental evidence supporting the origin of the hypothalamic decussations in the region of the pons or midbrain. According to Magoun & Ranson the majority of the fibres of the dorsal supra-optic decussation pass laterally through the hypothalamus into the region between the globus pallidus and the optic tract. They observed that some fibres enter the substance of the medial division of the globus pallidus and appear to end there, but most, after continuing caudally, are eventually scattered through the lateral part of the internal capsule whence they can no longer be traced. In our own sections numerous fibres, after crossing the ventral part of Ganser's commissure, end within the pallidum; the remainder turn medially around the lateral border of the internal capsule and their further course is discussed below.

(*b*) *Medial fillet and the contralateral thalamus.* As noted above, the medial fillet, while passing through the caudal region of the thalamus, sends a fair number of coarse degenerated fibres dorsally, which cross over in the posterior commissure to enter the opposite thalamus. The majority of these fibres end in the most medial part of the posterior ventral nucleus; but the posterior ventral nucleus of the thalamus contralateral to the lesions has still another source of fillet fibres, for the fibre tract, which has been described above as ending partly within the pallidum, sweeps around or pierces the internal capsule and terminates in the medial part of the ventral nucleus.

(*c*) *The corpus striatum and the hypothalamic commissures.* One of the most recent and important papers dealing with the relationship between these structures is by Ranson *et al.* (1941). Lesions were placed by them in various parts of the striatum in monkeys, and the brain sections stained by the Swank-Davenport modification of the Marchi method. Their preparations showed that the caudate nucleus gives rise to fibres which cross the internal capsule into the external division of the globus pallidus. Lesions in the caudate nucleus or putamen caused no degeneration of fibres within the ansa or fasciculus lenticularis. The authors hold the view that the pallidum is the only part of the corpus striatum which directly establishes connexion with the hypothalamus and thalamus. The fibre tracts connecting the nucleus caudatus with the pallidum were observed to run obliquely through the internal capsule into the globus pallidus. None of these fibres were found to cross the putamen in

order to reach the pallidum, despite the fact that in sections of normal brains a great number of fibres can be seen to pass from the caudate nucleus towards the putamen. Ranson *et al.* were aware of this arrangement of fibres in normal material and they do not exclude the possibility that only the minority of the degenerated caudato-fugal fibres were stained in their sections. But if this was the case it does raise some doubt as to the reliability of their Marchi technique, for in our own Marchi preparations we were able to see that very numerous fibres do in fact pervade the putamen (see Glees, 1944) to reach the pallidum. A fair number of these fibres, however, penetrate the putamen, and after breaking through the anterior commissure turn caudally and medially to form part of Ganser's commissure. It may also be noted that Kappers (1921), on the ground of his comparative studies, suggested that in mammals Ganser's commissure receives a contribution from the striatum. This connexion of the caudate nucleus is slightly anterior to the pallido-hypothalamic tract but mingles somewhat with its most anterior part. A further difference between the strio-hypothalamic connexion and the pallido-hypothalamic tract is that the former mostly undergoes decussation while the latter terminates mainly ipsilaterally (Ranson *et al.* 1941).

It would be beyond the scope of the present paper to given an extensive discussion on the subject of the supra-optic decussations, a field in which the neurological work of almost a century has led to so many divergent conclusions, but it is necessary to draw attention to the laborious work of Bodian (1940) on the opossum brain. In this study the supra-optic decussations have been dealt with in a careful examination of normal material. Bodian describes a decussatio supra-optica dorsalis, pars dorsalis (commissure of Ganser), decussatio supra-optica dorsalis, pars ventralis (commissure of Meynert), and a decussatio supra-optica ventralis (commissure of Gudden). He was also able to follow a smaller component of Ganser's commissure forming its ventral or rostral division, which at its place of crossing is mixed with the fibres of Meynert's supra-optic decussation. Instead of passing in a dorsal direction as the more dorsally situated fibres do, these fibres, according to Bodian, pass laterally and appear to enter the globus pallidus. It is clear from his description that this pathway is identical with the course of the degenerated fibres which we have found emerging from the medial fillet and passing into the globus pallidus of the opposite side. Differing from this course is the striatal connexion with the hypothalamus which is more precisely located within Ganser's commissure, or, to follow the more exact terminology of Bodian, within the decussatio supra-optica dorsalis, pars dorsalis (Text-fig. 3).



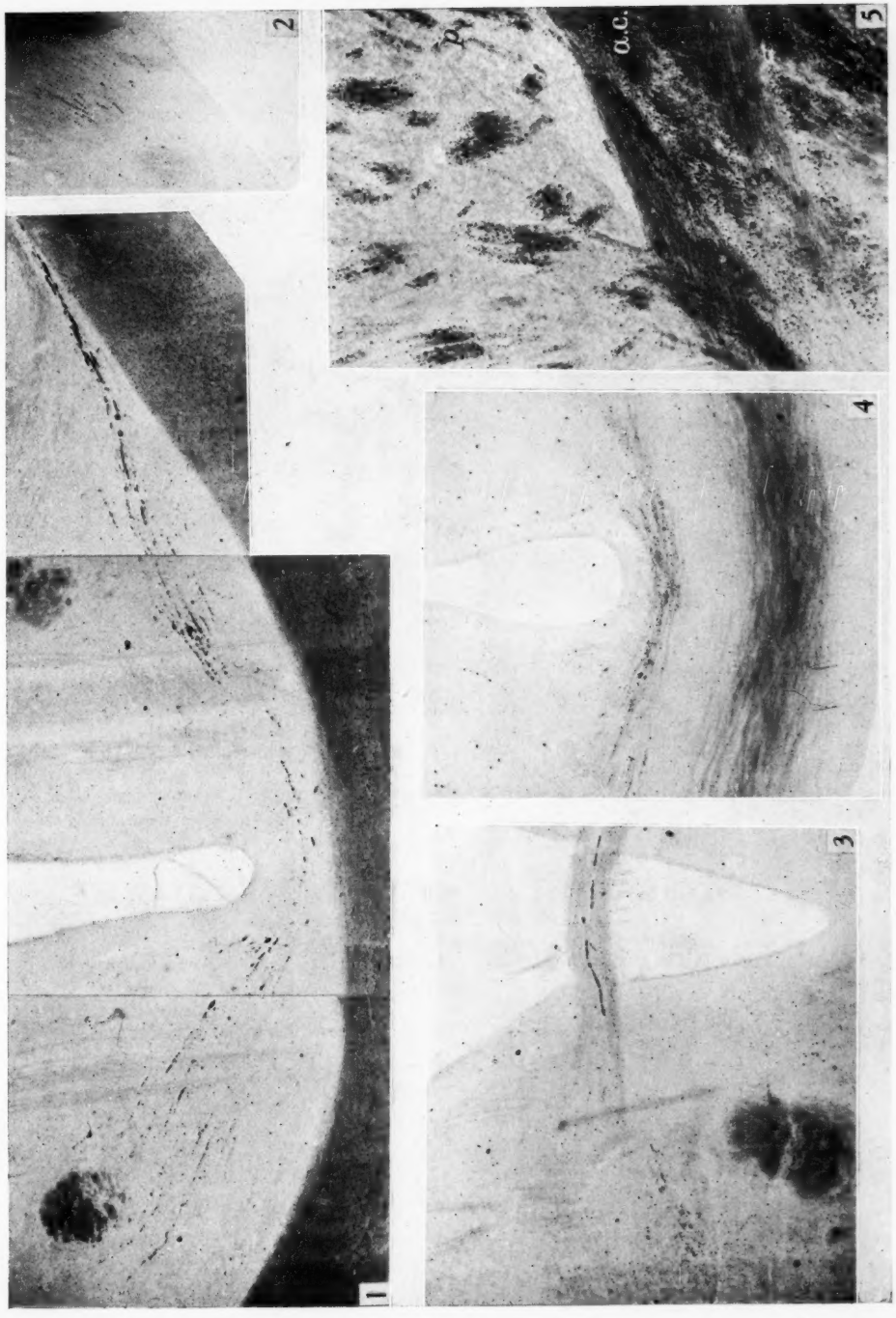
Text-fig. 3. A diagram illustrating the relative position of the supra-optic commissures in the cat. *o.t.* optic tract; *c.m.* commissure of Meynert; *G.v.* ventral part of Ganser's commissure; *G.d.* dorsal part of Ganser's commissure; III *v.* third ventricle; *c.i.* capsula interna; *p.* globus pallidus; *F.* descending pillar of fornix.

SUMMARY

1. A fibre connexion of the medial fillet with the opposite globus pallidus is described. The fibres pass through the ventral part of Ganser's commissure or decussatio supra-optica dorsalis.

2. The striatum is connected with the opposite hypothalamus via the dorsal part of Ganser's commissure or decussatio supra-optica dorsalis, pars dorsalis (Bodian, 1940).

3. Degenerated fibres of the lateral fillet were found to terminate within the posterior colliculus,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but none could be traced into the medial geniculate body.

The author wishes to express his thanks for a grant from the Nuffield Committee for the Advancement of Medicine and to Mr W. Chesterman for his technical assistance.

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EXPLANATION OF PLATE

Fig. 1. A composite picture showing the degeneration in the ventral part of Ganser's commissure following an experimental lesion in the medial fillet of the left side in cat 3 at the upper medullary level. Degenerated fibres from the left medial fillet are seen on the left side of the picture descending ventro-medially to pass beneath the third ventricle. On the right side the fibres pass laterally close to the dorsal part of the optic tract and thus contrast with the undegenerated fibres of the dorsal part of Ganser's commissure which run up into the hypothalamus. ×22.

Fig. 2. Section of the hypothalamus of a rabbit showing the degenerated fibres resulting from an injury to the caudate nucleus. They are seen entering the hypothalamus of the same side in close relation to the descending pillar of the fornix which can be seen faintly outlined. ×12.

Fig. 3. Section through the region of the posterior commissure showing the Marchi degeneration following lesions of the medial fillet. Degenerated fibres can be seen ascending from the medial fillet to the posterior commissure and decussating to reach the opposite thalamus. ×22.

Fig. 4. Section of the hypothalamus showing degeneration of fibres in the dorsal part of Ganser's commissure following a lesion of the caudate nucleus in a cat. Below Ganser's commissure the undegenerated fibres of the commissure of Meynert are shown. ×16.

Fig. 5. Section of the ventral part of the putamen showing the degenerated fibres resulting from a lesion in the caudate nucleus of a rabbit. The degenerated fibres cross the putamen and pierce the anterior commissure, finally to end partly in the amygdala of the same side and partly in Ganser's commissure. ×49. *p.* putamen; *a.c.* anterior commissure.

OBSERVATIONS ON THE HISTOLOGY OF THE CHOLEDOCHO-DUODENAL JUNCTION AND PAPILLA DUODENI, WITH PARTICULAR REFERENCE TO THE AMPULLA OF VATER AND SPHINCTER OF ODDI

By JOHN KIRK, *Department of Anatomy, Middlesex Hospital, London*

The observations here recorded were made while investigating the exact site and disposition of muscle fibres at the entrance of the biliary and pancreatic ducts into the duodenum.

MATERIAL AND METHOD

Except in one instance, only human material was examined. The latter consisted of seven adult specimens obtained post-mortem, two full-time still-born foetuses and one 6-months foetus. Serial sections of a 27 mm. human embryo were also examined as well as sections of the choledocho-duodenal junction in an adult cat. The autopsy material was fixed in formol-saline or Bouin and microscopical preparations were stained with haematoxylin and either the Van Gieson or Ponceau S. methods.

OBSERVATIONS

The ampulla of Vater. As is now well known, a dilated or flask-like ending of the united ducts in man is frequently of diminutive size or even absent. Giordano & Mann (1927) have recorded that in material obtained at autopsy, 76 % of cases showed an ampulla of only 2 mm. or less in length. In all of our series of seven adult specimens no ampulla, in the accepted meaning of the term, was found, but in each the papilla duodeni was well defined. In one of the two full-time foetuses a well marked ampulla was present.

Papilla duodeni. The functional significance of the papilla is obvious when its internal structure is examined. Here the mucosa of the united duct forms a number of valvular folds or ridges which divide the lumen into a labyrinth of recesses communicating with the main channel. In cross-section, the appearance is strikingly similar to the ampullary portion of the oviduct (plicae ampullares). The words used by Maximow & Bloom (1943) to describe this might well be used in connexion with the papilla (Pl. 1, figs. 1, 2). It 'looks like a labyrinth of narrow spaces between epithelium-lined partitions'. These valvular folds possess a core of connective tissue in which muscle fibres are frequently present and the arrangement suggests a mechanism well adapted to prevent flow otherwise than towards the intestine.

In the papilla the rich supply of mucous glands characteristic of the biliary tract is even more accentuated. In the full-time foetus the epithelial covering of the duodenal mucosa in the region of the ostium is found to be interspersed with many goblet cells (Pl. 1, fig. 3).

Disposition of muscle.

The extra-duodenal part of the bile duct is devoid of muscle except in close proximity to the point where it penetrates the gut wall. Here longitudinal fibres are found passing with the duct through the longitudinal coat of the bowel. A thickening of the circular coat of the duodenum at the site of penetration is very noticeable and an associated enlargement of Auerbach's plexus suggests a special functional value of the intestinal muscle in this region related to the terminal portion of the duct. In the cat this thickening of the circular coat (as much as three-fold) and the increase in size of Auerbach's plexus are very striking (Pl. 2, figs. 4, 5). In fig. 5 the common bile and pancreatic ducts, which have not yet joined, can be seen surrounded by muscle continuous with the circular coat of the intestine. In the human material the submucosal portions of the ducts are surrounded by circularly disposed muscle and these sphincteric fibres are likewise continuous with the circular coat of the intestine (fig. 6). As the sphincter muscle is traced towards the papillary region some of the fibres assume an oblique direction and enter the valvular fringes of the mucous membrane.

In none of the specimens examined was any trace of muscle found at the ostium.

From this it would appear that the contraction of the circular muscle of the bowel and of the muscle surrounding the submucosal portion of the bile duct not only exerts a sphincteric or closing effect by compression of the duct wall but, by means of the prolongation of its fibres into the valvular folds, also exerts a constringing and retracting effect upon the latter, bunching them together in a manner calculated to occlude the lumen of the papilla.

Serial sections of the duodenum in a 27 mm. human embryo suggest that circularly disposed fibres are present in the intraduodenal portion of the duct wall at a very early stage, and that they are continuous with the circular muscle differentiating in the wall of the intestine (Pl. 3, fig. 7). Admittedly, this is not in harmony with the view of Schwegler & Boyden (1936, 1937) who hold that the

sphincter mechanism of the duct and the circular muscle of the gut are separate entities.

In the submucosal portion of the bile duct mucous crypts are abundant and diverticula of its wall are numerous. Diverticula of the extra-duodenal part of the bile duct sometimes penetrate the muscle wall of the duodenum from without and end blindly in the submucosa. These pockets are also rich in mucous glands and are often themselves surrounded by a coat of circularly disposed muscle (Pl. 3, fig. 8).

Relation of pancreas to duodenal wall. In one of our adult specimens a lobule of pancreatic tissue, continuous with the main gland, was found lying between the longitudinal and circular coats of the intestine (Pl. 3, fig. 9). This condition would account for those cases in which, when mobilizing the duodenum, the surgeon experiences difficulty in separating the head of the pancreas without injury to the duodenal wall.

Nervus choledochus. In support of the view that a neuro-muscular mechanism is responsible for the closure and opening of the terminal portion of the common duct, we have noticed a large nerve trunk running in close apposition to the extra-duodenal portion of the biliary tract as far as the point where it penetrates the duodenal wall. Adult specimens as well as full-time foetuses show this to be a prominent structure (Pl. 3, fig. 10) and the large size of Auerbach's plexus in this situation, already referred to, is suggestive.

Chiu (1943) mentions that Johnson & Boyden have recently 'demonstrated that in the cat the dorsal vagus nerve supplies the nerves which relax the choledocho-duodenal junction', and it would not be surprising to find that such a vagal distribution has its counterpart in man.

A further study is being made of nerve cells and fibre endings in the choledocho-duodenal area with a view to determining whether or not this particular nerve bundle merits the title 'nervus choledochus'.

SUMMARY

The histology of the extra-biliary tracts has been studied in embryo, foetal and adult human material. In a series of seven adult specimens no Vaterian ampulla was found. In one of two full-time foetuses, however, a well-marked ampulla was present. In the adult specimens the papillary portion of the united bile and pancreatic ducts appeared as a narrowing channel packed with valvular folds of its mucous membrane well adapted to prevent regurgitation.

The sphincter of Oddi was represented by circular muscle fibres not at the ostium, but surrounding the submucosal portion of the duct proximal to the papilla and continuous with a thickened circular coat of the duodenum at the site of penetration. Auerbach's plexus was noticeably enlarged in this part of the intestinal wall. The extension of muscle fibres into the connective tissue of the valvular folds of the papillary region suggests a constringing and retracting action as well as a sphincteric function of the muscle of Oddi, consistent with closure of the lumen of the submucosal portion of the bile duct and of the papilla, but there is no sphincter muscle at the ostium.

The configuration of the papillary lumen readily explains the obstructive effect of inflammatory swelling in this region, as, for example, in cases of catarrhal jaundice. The common bile duct has a substantial companion nerve. The occasional presence of extensions of pancreatic tissue between the two muscle layers of the duodenum would account for the difficulty sometimes experienced by the surgeon in separating the duodenal wall from the head of the gland.

For the very helpful co-operation and stimulating enthusiasm of my friend, Surgeon Rear-Admiral Gordon Gordon-Taylor, at whose suggestion this study was undertaken, my warmest thanks are due.

I am also greatly indebted to Mr L. Carnac Rivett and members of the Staff of the Middlesex Hospital Obstetric and Gynaecological Department, to Prof. J. McIntosh and members of the Staff of the Bland-Sutton Institute, and to Prof. M. J. Stewart of the Leeds University Medical School, all of whom have very kindly supplied me with material for these investigations.

To Mr S. R. Scarfe, of my own Department, who has carried out the laborious task of preparing the histological sections and micro-photographs with his usual fidelity and skill, my grateful acknowledgment is also due.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Duodenal papilla of human adult. Transverse section. ×12. Common duct lying in submucous coat of bowel, approaching ostium.

Fig. 2. Duodenal papilla and ostium of human adult. Longitudinal section. ×11.

Fig. 3. Ostium in full-time human foetus. Longitudinal section. ×122.

PLATE 2

Fig. 4. Duodenal papilla, Felis domesticus. Longitudinal section. ×13. Note thickening of circular muscle of intestine in this region.

Fig. 5. Duodenum of cat. Transversesection. ×10·5. Note common bile and pancreatic ducts completely surrounded by muscle continuous with circular coat of intestine.

Fig. 6. Biliary and pancreatic ducts in submucosa of duodenal wall. Human foetus, 6 months. Longitudinal section. ×30. Note muscle surrounding ducts and continuous with circular coat of bowel.

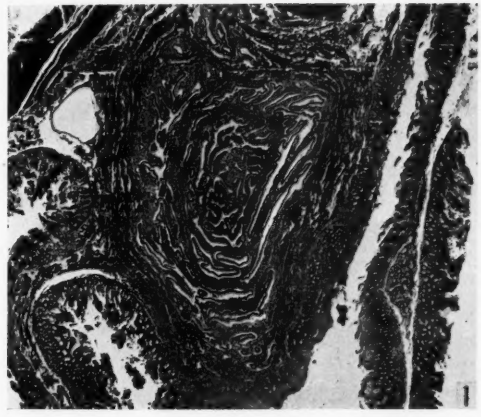
PLATE 3

Fig. 7. Duodenum, human embryo. 27 mm. Transverse section. ×50. Note common bile duct and pancreatic ducts in submucosa surrounded by differentiating muscle continuous with muscle of duodenal wall.

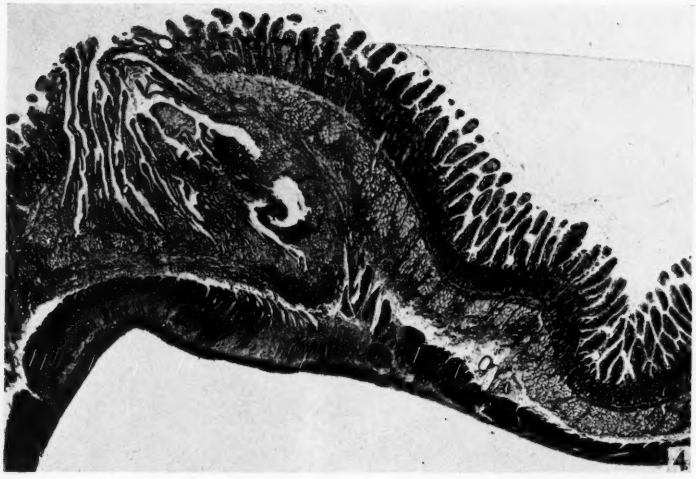
Fig. 8. Duodenal wall. Human adult. Longitudinal section. ×38. Note diverticulum of common bile duct penetrating muscle wall of duodenum from without.

Fig. 9. Duodenal wall. Human adult. Longitudinal section. ×28. Note pancreatic tissue lying between longitudinal and circular muscle of intestine.

Fig. 10. Extra-duodenal common bile duct. Human foetus. 6 months. Longitudinal section. ×50. Note large nerve trunk (*N*) in juxta-position to duct (*C.B.D.*).



KIRK—HISTOLOGY OF THE CHOLEDOCHO-DUODENAL JUNCTION AND PAPILLA DUODENI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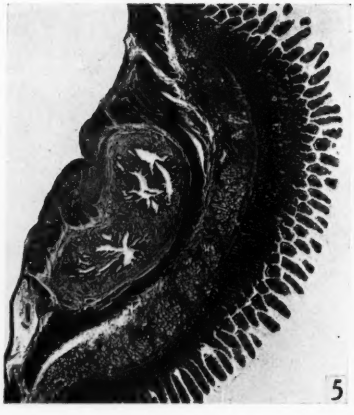
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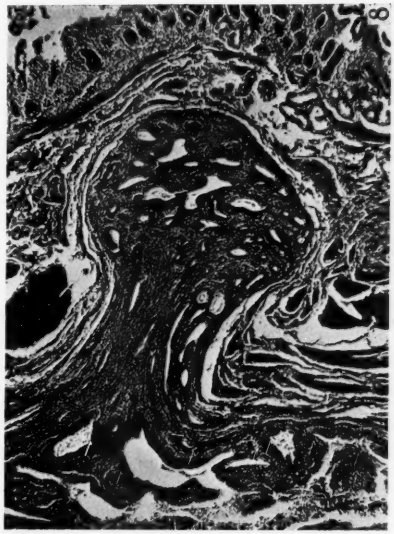
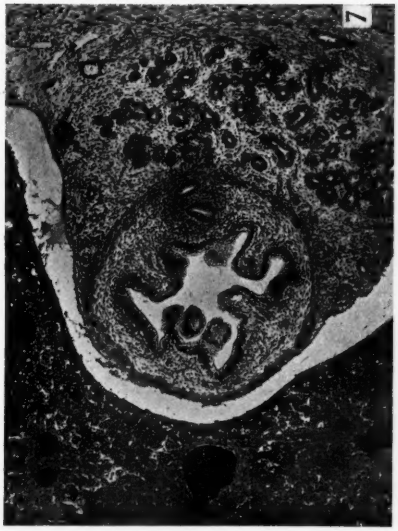
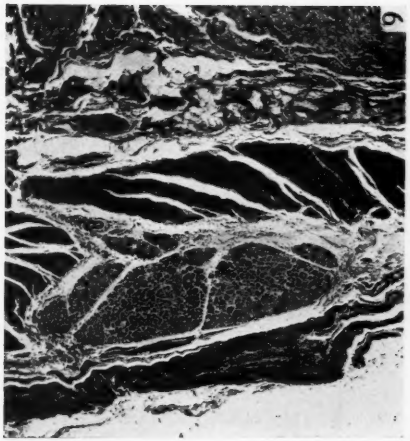






UNIV. OF MICH.

THE EFFECT OF 1:2:5:6-DIBENZANTHRACENE ON THE LYMPH-NODES OF THE RAT

By A. LASNITZKI and D. L. WOODHOUSE. *From the Department of Physiology and the Department of Pathology, The Medical School, University of Birmingham**

A brief description has been given previously (Lasnitzki, 1938) of changes observed in the lymph-nodes of rats which had received subcutaneous injections of carcinogenic tar. Macroscopically, these consisted of red-brown discolorations varying in extent and degree. Microscopical examination revealed that the lymph-nodes had been converted into more or less pronounced haemolymph-nodes characterized by the occurrence of extravascular red blood corpuscles, and a general diminution of the normal cellular constituents, yet prominence of a comparatively large pigmented cell type. This structural alteration was frequently noticeable in certain distinct areas.†

Since the animals employed in these experiments showed a progressive deterioration in general health, the question arose whether the changes were due entirely to substances with purely toxic effects, or whether they were produced, at least in part, by those specific constituents responsible for the carcinogenic action of the tar. The investigations were therefore continued with a pure carcinogenic hydrocarbon, 1:2:5:6-dibenzanthracene, likewise injected subcutaneously into rats. The effects have been studied in more detail and are described in the present paper. For 1:2:5:6-dibenzanthracene the abbreviation 'd.b.a.' will frequently be used in the text.

Preparation of a colloidal solution of 1:2:5:6-*dibenzanthracene.* With the aim of subjecting the body throughout several weeks to a small but controlled and continuously renewed quantity of carcinogen, it was decided to introduce the hydrocarbon in the form of an aqueous colloidal solution.‡ A solution containing 0·001 mole hydrocarbon per litre was employed, prepared by the following convenient method: 27·8 mg. of d.b.a. (Hoffmann-La Roche) were dissolved in a small amount of acetone. 100·0 c.c. of a solution of 5·6 % glucose and 0·5% gelatine (approximately isotonic to rat serum) were then heated on a water-bath, and the acetone solution of the hydrocarbon was added slowly, with constant stirring. The acetone was completely evaporated by warming, the liquid cooled and its volume restored to exactly 100·0 c.c. with distilled water. It was divided equally among a number of tubes, so that the content of one tube was sufficient for the series of injections made on one day. After steam sterilization the tubes were stored in a refrigerator. The colloidal solution thus prepared was fairly stable, although a small amount of a fine precipitate was noticed after standing some time. This was distributed homogeneously in the liquid before making the injection. A solution for injecting control animals was prepared by adding acetone only to the glucose-gelatine vehicle and continuing the preparation exactly as for the colloidal solution.

Arrangement of experiments. Pre-adult male rats, of a mixed strain and various colours, were employed and given a diet of white bread and oats. The initial body weight was usually between 100 and 150 g. Three main experiments were performed, using an equal number of control and d.b.a.-treated animals in each, a total of eight in Exp. I and of sixteen in each of Exps. II and III. One subcutaneous injection of 1·0 c.c. of the appropriate solution was given into alternate flanks, as a rule on five consecutive days in each week. The maximum number of injections per animal was twenty-two in Exps. I and II, and twenty in Exp. III. The weight of every animal was recorded weekly. From time to time one or two animals were selected from the control and d.b.a. series, killed with chloroform on the same day and dissected. The animals of each pair usually had similar initial weights. A proportion of the animals (i.e. three pairs in Exp. I, five in Exp. II, two in Exp. III) was examined before, the remainder after the full number of injections was given.

General health and growth of animals. During the experiments the animals remained in good general health and continued to grow. Accidental disorders occurred very rarely. The rate of growth varied considerably among individual animals; but it was, on an average, clearly less among the animals injected with d.b.a. than among the controls. This will be seen from Text-fig. 1, which shows the average percentage increase in body weight of eight test animals and eight corresponding controls for the whole period of treatment. The total increase was about 25 % in the former group compared with

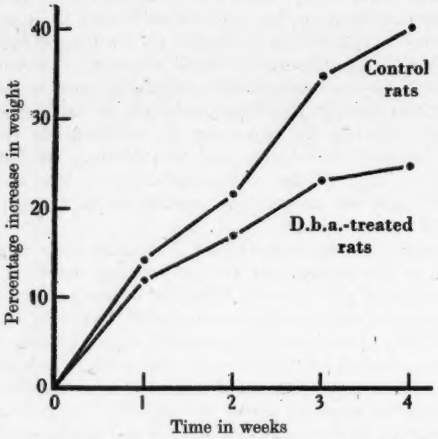
* Part of the work was carried out at the Cancer Research Department, University of Manchester.

† For a general account of our knowledge of haemolymph-nodes in animals and man, under normal and pathological conditions, see the review by Weller (1938).

‡ The use of aqueous colloidal solutions of carcinogenic hydrocarbons in experimental work has been initiated independently by Berenblum (1932) and Boyland (1932).

about 40 % in the latter, the difference being significant, although the d.b.a.-treated animals started with a slightly higher absolute value. A similar inhibition was observed among other d.b.a.-treated animals whose weights were followed up only until the end of the third week. During the third and fourth weeks of treatment, as well as during the period following the injections, individual members showed a complete arrest of growth, or even a slight decrease in weight.

This inhibitory effect agrees with similar findings of Haddow, Scott & Scott (1937) and Lees (1937*a*, 1937*b*), obtained under somewhat different conditions.



Text-fig. 1. Percentage increase in body weight of rats injected with control solution and d.b.a. solution respectively. Average values from eight animals in each group. Average of initial absolute weights: control rats 119·0 g.; d.b.a.-treated rats 126·5 g.

Post-mortem examination of lymph-nodes. The lymph-nodes were removed from various regions of the body and examined macroscopically. According to their location the following nodes were distinguished:

Submaxillary n.	Thoracic n.
Cervical n., l. and r.	Mesenteric n.
Axillary n., l. and r.	Renal n.
Inguinal n., l. and r.	Lumbar n.
Popliteal n., l. and r.	Caudal n.

The number of nodes present in the cervical, axillary and inguinal regions was usually two, in the popliteal region one on each side. There were usually two nodes each in the renal and lumbar region, and one in the caudal region. The number of nodes in the submaxillary region varied approximately between six and ten, in the thoracic region between four and six, and in the mesenteric region between eight and twelve. The size of the nodes varied to some extent from animal to animal, but more so from region to region of the same animal and within the same region, the greatest intra-regional variation being among the group of mesenteric nodes. The largest nodes were usually found in the mesenteric and submaxillary regions, the smallest in the mesenteric and thoracic regions. After the macroscopical characteristics of the lymph-nodes had been recorded, a proportion of them was fixed in formol-saline. Paraffin sections were cut and a representative number of these was stained with haematoxylin-eosin.

THE CHANGES OF THE LYMPH-NODES, INDUCED BY 1:2:5:6-DIBENZANTHRACENE

A. *The colour changes*

The lymph-nodes of the animals that had been injected with the control solution were generally of a light yellowish colour. At times a darker yellow shade was observed. Thus there was little difference between the colour of these nodes and of those from completely untreated animals. In contrast, the lymph-nodes of the animals that had been injected with the d.b.a. solution frequently showed a red or brownish red discoloration varying in extent and degree. A record of these findings for the whole of the animals concerned is given in the accompanying chart (Pl. 1), which also sets out for each pair of rats the number of injections as well as the number of days elapsed, at the date of killing, since the first and the last injection. The lymph-nodes in ten different regions of the body are considered, each individual node being represented by a small oval area. Nodes with normal colour are indicated by unshaded areas, discoloured nodes by shaded areas, for which purpose four grades of shading have been chosen.* The pronounced difference in the appearance of the nodes from control and d.b.a.-treated animals is clearly visible, a considerable change being evident in many of the latter nodes. The popliteal and caudal nodes are not represented in the chart; the colour changes shown by them were, for the most part, similar to those observed among the inguinal and lumbar nodes. The renal nodes, which also are not represented, will be considered in a special section.

The extent of discoloration varied greatly in nodes from different regions of the same animal and also in those from corresponding regions of different animals, and such variations were apparently unconditioned by the initial body weight. Further,

* The number of areas representing the submaxillary, thoracic and mesenteric nodes corresponds approximately to the average number of nodes found in these regions, the variation in size being compensated for as far as possible; while the nodes in each of the remaining regions are, in accordance with their normal number, assigned two areas.

the size of the discoloured area and the intensity of colour change therein frequently varied among individual nodes of the same region. In many cases the discoloration mainly occurred at the periphery, but commonly the change was present over portions of one or both surfaces of the node, often involving the whole of both surfaces. Some specially conspicuous nodes were encountered in which one surface was completely discoloured, while the other one looked almost normal. The colour intensity showed all transitions from a slightly reddish to a dark brownish red tone, and an increase in area of discoloration tended to be associated with an increase in that intensity. On the other hand, certain nodes were found which showed a general reddish discoloration over one or both surfaces, while others mainly had a dark brownish red discoloration at the periphery. As far as possible, this two-fold variation has been illustrated by the method of shading adopted in the diagrammatic representation of the nodes.

In the smaller experiment (I) little correlation was apparent between colour change and duration of treatment. However, in Exp. II, in which each pair of rats (except the last) was killed 1 day after the final injection, the amount of discoloration tended to be greater at the later stages of treatment. Rat 8 (d.b.a.-treated group), which was killed 15 days after having received the full number of injections, still showed considerable changes, indicating that after the conclusion of prolonged treatment the discoloration of the nodes might last for at least 2 weeks. This was further investigated in Exp. III, in which only two animals of each group were killed before receiving the full number of injections, while six others were killed at intervals of 6–14 days after that period. It will be seen that the changes were, as a whole, even more pronounced 12–14 days after the conclusion of treatment. In particular, the axillary, inguinal and lumbar nodes were more than usually affected at that late stage.

B. *The histological changes*

Structure of lymph-nodes from control animals. In sections of lymph-nodes from control animals a cortical and a medullary part could be distinguished. The relative disposition of these appeared to vary, but in suitable sections the cortex could be observed as a zone surrounding the medulla to a greater or lesser extent, except in the region of the hilus. In other instances the medulla seemed to form a narrow strip along almost the whole of one surface, the rest of the node being occupied by the cortex. (These two kinds of arrangement may be correlated with the two types of lymph-nodes observed in the common wild rat by Job (1922), although the structural features of type II as described by this author have, so far, not been noticed in our sections.) The cortex consisted essentially of dense lymphatic tissue with a number of more or less circumscribed nodules in which 'germinal centres' were usually present. The medulla contained the central lymph sinus traversed by anastomosing cords of relatively loose lymphatic tissue which were continuous with that of the cortical region. The entire node was enclosed in a thin fibrous capsule, separated—at least partially—from the lymphatic tissue proper by the marginal lymph sinus. In general this was a narrow space, but wider portions were observed, especially close to the periphery of the node where the marginal sinus was about to join the central sinus, and also in such areas where smaller lymph paths originated apparently connecting one sinus with the other. Small blood vessels and capillaries were noticed fairly often in the cortex and in the medullary cords, but were seen more rarely in the lymph spaces.

The main cellular constituents of the cortex were lymphocytes, predominantly of small size, and lymphoblasts, the specific cells of the 'germinal centres'. The lymph cords of the medulla consisted mainly of lymphocytes and plasma cells. The medullary lymphocytes were generally larger than those of the cortex, but intermediary forms akin to the plasma cells were visible so that a clear distinction was not always possible. A varying number of lymphocytes, mostly of the small type, were present in the central sinus, the marginal sinus and the connecting lymph paths. These lymph spaces also contained the reticular cells, characterized by their relatively large but variable size, irregular shape and, as a rule, faintly stained nucleus. The marginal sinus and the connecting lymph paths usually showed many more reticular cells than the central sinus. In this latter region great variation in number was encountered, and they were represented at times by a few scattered individuals only. In many instances, especially when lying singly or in loose clusters (principally, in the central sinus), the reticular cells seemed to be attached to fine fibrils which often showed a net-like arrangement. Moreover, when present in dense aggregates (marginal sinus, etc.) the cells tended to form a syncytium with frequent indication of distinct protoplasmic connections. A limited number of reticular cells, especially in the central sinus, appeared to be converted into free macrophages, recognizable by their complete segregation and rounded shape. Occasionally they contained some pigment or other particulate matter. Varying numbers of reticular cells were also observed in the lymphatic tissue of the cortex as well as of the medulla. Although many red blood corpuscles were often seen inside the blood vessels and capillaries, they occurred outside these channels in very few instances only, when small numbers of extravascular corpuscles could be noticed, lying singly or in little groups.

A comparative study of corresponding lymph-nodes from control animals and completely untreated animals did not reveal any appreciable difference in structure. Thus the injection of the control solution was without observable influence.

Structure of lymph-nodes from d.b.a.-treated animals. In describing the structure of lymph-nodes from d.b.a.-treated animals it is necessary to distinguish between those which were of normal colour and those which showed various degrees of discoloration. The microscopic appearance of the former nodes was essentially the same as that of the nodes from control animals. On the other hand, obvious changes were apparent in the structure of the discoloured nodes, differences which could be correlated with the amount of macroscopic alteration. The principal features were: (1) widening of the lymph spaces and diminution of the lymphatic tissue; (2) presence in the lymph spaces of red blood corpuscles; (3) increase in the number of free macrophages carrying red blood corpuscles and pigment.

In lymph-nodes that had shown only slight superficial discoloration usually the number of abnormally situated red blood corpuscles was small. As a rule, a limited area of the marginal sinus appeared to be involved; in particular, the wide peripheral portion of this space mentioned previously was often affected. The latter localization is seemingly associated with the circumscribed discoloration so often observed at the periphery of these nodes. In nodes with intense discoloration the numbers of corpuscles in the lymph spaces were much greater. In many such nodes a large portion of the marginal sinus appeared to be widened, and lymph paths connecting the marginal with the central sinus were observed more frequently than in the corresponding nodes of control animals. At very advanced stages the entire node was traversed by a plexus of wide lymph channels, which were separated by scanty lymphatic tissue usually having the structure of medullary cords, while the amount of cortex was reduced not only absolutely but also relatively. However, the detailed structure of the two portions of tissue showed little deviation from normal. The red blood corpuscles which were so distinctive a feature were sometimes distributed throughout the lymph spaces and sometimes confined to certain portions of them. The density of aggregation varied in different nodes as well as in different parts of the same node. In some instances the density was greatest in the marginal sinus and adjoining lymph paths, the central sinus having only a small number. In others the central sinus showed the highest concentration of corpuscles. Both these appearances are probably related to the occurrence of many nodes in which a pronounced discoloration was confined almost entirely to one surface. In general, very few or no corpuscles were observed inside the lymphatic tissue, even when they were aggregated fairly densely in the adjacent lymph spaces. This fact seems to exclude any relation which the corpuscles might have to the blood vessels and capillaries of the lymphatic tissue.

The lymph spaces also contained lymphocytes and reticular cells, both of which varied in size and number. While the lymphocytes were always normal in appearance, striking changes were observed in the reticular cells of the affected regions of the node. Many of these cells appeared to be converted into free macrophages, their number being greatly increased as compared with the small number usually seen in unaffected regions and in nodes from control animals. The macrophages showed certain distinct features which were also noticed in some of the ordinary reticular cells, although a clear distinction between these and macrophages proper was not always possible. A proportion of the cells contained one or several red blood corpuscles, apparently taken up by phagocytosis. Frequently the entire cell body was full of corpuscles, or groups of slightly coloured vacuoles indicated the presence of corpuscle residues. Moreover a large number, if not the majority, of the cells was loaded with a pigment, present in the form of either fine particles or coarser granules. It was usually of a distinct greenish-brown colour, with certain variations in shade. Broadly speaking, the extent and degree of these cellular changes increased with the number of free red blood corpuscles present in the lymph spaces. In the marginal sinus, however, the changes tended to be less than in the other lymph spaces, even when corpuscles were present in similar density. Many free macrophages containing red blood corpuscles and much pigment were often observed in the central sinus, although here, as a rule, they were less densely aggregated than in the adjacent parts of the narrower lymph channels that connected the central and marginal sinuses. Along these channels their frequency tended to increase towards the central sinus. Also a varying number of free macrophages (and evidently ordinary reticular cells), often with a considerable quantity of pigment, were found in the lymphatic tissue, especially in areas bordering the lymph spaces. In sections that showed advanced stages of change the entire number of free pigment-carrying macrophages was fairly large, while the free red blood corpuscles were relatively less abundant. This condition might explain the deep brownish tone in the colour of these nodes.

C. *Demonstration of free iron*

The histological features reported in the previous section suggest that a process of erythrocytolysis occurs in many lymph-nodes of the d.b.a.-treated rats: red blood corpuscles are taken up by phagocytosis into free macrophages and ordinary reticular

cells and undergo therein a process of destruction in course of which the haemoglobin is decomposed chemically. It would appear that the intracellular pigment described above is the first noticeable product of this decomposition. From consideration of well-known facts it was concluded that the pigment was 'haemosiderin', a material characterized by its content of free iron. In order to test this assumption it was regarded as desirable to supplement our investigations by a microchemical study of free iron detectable in lymph-nodes from d.b.a.-treated and control animals. For this purpose a special experiment was carried out, under conditions similar to those of the general experiments hitherto considered.

Four preadult male rats were injected with the d.b.a. solution, and four others with the control solution. In both groups of animals the body weights increased till the end of the second week of treatment, the average increase being somewhat smaller in the d.b.a.-treated series. During the third and fourth weeks, however, the average weights showed little change, although the general health of the animals remained satisfactory. One pair of control and d.b.a.-treated animals was killed 3 days after the 18th injection, the other pairs 7, 13 and 17 days respectively after the 20th injection. While the lymph-nodes from the control animals were of normal colour, a proportion of the nodes from d.b.a.-treated animals showed distinct colour changes, comparable with those described above. In the last two animals the changes were, on the whole, more pronounced than in the two animals examined earlier. From each d.b.a.-treated animal a number of nodes showing various degrees of discoloration, and from each control animal a similar number of corresponding nodes, were prepared for microscopical examination (fixation in absolute alcohol; preparation of frozen or paraffin sections). The free iron was precipitated according to the Turnbull blue method of Tirmann and Schmelzer, modified by Liesegang, and the sections were counterstained with carmin.

In their general histological structure, the discoloured lymph-nodes of the d.b.a.-treated animals showed the changes previously described. The results of the microchemical tests for iron were as follows. In the majority of the discoloured nodes the occurrence of iron could be clearly demonstrated. The amount varied considerably and, in general, appeared to run parallel to the intensity of the morphological changes, being very small or nil in slightly discoloured nodes and fairly large in nodes with pronounced discoloration. Very little or no iron could be detected in the nodes of control animals. The precipitate usually was observed as an inclusion within the free macrophages and ordinary reticular cells, situated in the lymph spaces or (less regularly) in the adjacent lymphatic tissue. Inside the cell body it occurred in the form of varying amounts of fine particles or coarser deposits. The particles were mostly of dark blue colour and, as a rule, were surrounded by a light-bluish stained protoplasm, probably due to a secondary process of dissolution. Frequently, however, the whole cell body, or the greater part of it, showed a fairly homogeneous dark blue stain, thus indicating a large accumulation of iron. When present in a moderate amount, the precipitate was often accompanied by clearly visible red blood corpuscles or their residues. The investigation showed that free iron was present in just those types of cells which contained the characteristic greenish brown pigment, and that the general distribution of the iron precipitate corresponded approximately to that of the pigment. The latter must, therefore, be regarded as 'haemosiderin', formed by chemical decomposition from the haemoglobin of the phagocytosed red blood corpuscles.

D. *Photomicrographs*

The histological changes observed in discoloured lymph-nodes are illustrated by seven pairs of photomicrographs (Pl. 2 and Pl. 3, figs. 9–14; magnification 450×). They were taken from sections through corresponding lymph-nodes of control and d.b.a.-treated rats, belonging to the same experimental series and killed either on the same or on successive days. Macroscopically, all the lymph-nodes selected from the d.b.a.-treated rats showed pronounced surface discoloration. In six pairs of photos the cellular structures of portions of the central sinus or adjoining lymph paths are the main objects of comparison, while in one pair the appearances of portions of the marginal sinus are compared. Particulars concerning the location of the nodes, treatment of the animals, and the details observable in the photographs are given in the following list of figures contained in Pls. 2 and 3:

Fig. 1. Submaxillary node of control rat. Twenty injections; killed 39 days after first, 12 days after last injection (cf. Pl. 1, Exp. III, no. 6). Part of central sinus showing lymphocytes of different sizes and reticular cells. Medullary cord formation indicated.

Fig. 2. Submaxillary node of d.b.a.-treated rat. Twenty injections; killed 41 days after first, 14 days after last injection (cf. Pl. 1, Exp. III, no. 8). Part of central sinus showing red blood corpuscles (most of them in great density), lymphocytes, and a number of free macrophages with large amounts of pigment.

Fig. 3. Cervical node (right) of control rat. Twenty injections; killed 41 days after first, 14 days after last injection (cf. Pl. 1, Exp. III, no. 8). Part of central sinus with lymphocytes, reticular cells and fibrils. In the centre a transverse section through a small medullary cord.

Fig. 4. Cervical node (right) of d.b.a.-treated rat. Twenty injections; killed 41 days after first, 14 days after last injection (cf. Pl. 1, Exp. III, no. 8). Part of central sinus with many red blood corpuscles (partly in great density), lymphocytes, and a number of reticular cells and free macrophages containing pigment and corpuscles (or c. residues).

Fig. 5. Cervical node (right) of control rat. Twenty injections; killed 33 days after first, 6 days after last injection (cf. Pl. 1, Exp. III, no. 4). Part of marginal sinus (inosculation of a cortical lymph path) with lymphocytes and reticular cells, the latter apparently forming a syncytium.

Fig. 6. Cervical node (right) of d.b.a.-treated rat. Twenty injections; killed 39 days after first, 12 days after last injection (cf. Pl. 1, Exp. III, no. 6). Part of marginal sinus with many red blood corpuscles, lymphocytes, and a number of reticular cells or free macrophages, some of them slightly pigmented.

Fig. 7. Thoracic node of control rat. Twenty injections; killed 35 days after first, 8 days after last injection (cf. Pl. 1, Exp. III, no. 5). Part of central sinus with some lymphocytes and reticular cells, located between one small and two large medullary cords consisting of lymphocytes, plasma cells and reticular cells.

Fig. 8. Thoracic node of d.b.a.-treated rat. Twenty injections; killed 35 days after first, 8 days after last injection (cf. Pl. 1, Exp. III, no. 5). Part of central sinus with many red blood corpuscles, lymphocytes, and a number of pigmented reticular cells and free macrophages.

Fig. 9. Mesenteric node of control rat. Twenty injections; killed 28 days after first, 1 day after last injection (cf. Pl. 1, Exp. II, no. 5). Part of a lymph path adjoining central sinus, with lymphocytes and reticular cells, the latter forming a syncytium.

Fig. 10. Mesenteric node of d.b.a.-treated rat. Twenty injections; killed 28 days after first, 1 day after last injection (cf. Pl. 1, Exp. II, no. 5). Part of a lymph path adjoining central sinus, with red blood corpuscles (most of them in great density), lymphocytes and a number of free macrophages containing corpuscles (or c. residues) and pigment.

Fig. 11. Mesenteric node of control rat (same node as in Pl. 3, fig. 9). Part of central sinus showing a network of reticular cells and fibrils (consisting of two parts), and lymphocytes.

Fig. 12. Mesenteric node of d.b.a.-treated rat (same node as in Pl. 3, fig. 10). Part of central sinus showing a number of free macrophages with varying amounts of pigment, a few slightly pigmented reticular cells, some red blood corpuscles, and lymphocytes.

Fig. 13. Lumbar node of control rat. Twenty injections; killed 28 days after first, 1 day after last injection (cf. Pl. 1, Exp. III, no. 3). Part of a lymph path adjoining central sinus, with some lymphocytes, reticular cells and fibrils, and parts of two medullary cords separated by a side path.

Fig. 14. Lumbar node from a d.b.a.-treated rat. Twenty injections; killed 33 days after first, 6 days after last injection (cf. Pl. 1, Exp. III, no. 4). Part of a lymph path adjoining central sinus, with many red blood corpuscles (mostly in great density), lymphocytes, and a number of free macrophages, containing pigment and corpuscles residues.

(Sections for Pl. 2 and Pl. 3, figs. 9–14, stained with H.+E.)

The occurrence of free iron in lymph-nodes which showed intense discoloration is illustrated in two additional photomicrographs (Pl. 3, figs. 15, 16; magnification as above). The particulars are:

Fig. 15. Cervical node (left) of d.b.a.-treated rat. Twenty injections; killed 44 days after first, 17 days after last injection. Part of central sinus and adjacent part of a medullary cord, showing two free macrophages completely loaded with iron precipitate.

Fig. 16. Lumbar node of d.b.a.-treated rat. Twenty injections; killed 40 days after first, 13 days after last injection. Part of central sinus showing a number of free macrophages with much iron precipitate; in some of them a few red blood corpuscles, or their residues, are indicated.

SPECIAL FINDINGS ON LYMPH-NODES OF CONTROL ANIMALS

Among all the lymph-nodes examined, the renal nodes (situated medial of the kidneys) were exceptional since already those from control animals usually showed a brownish red coloration. This occurred sometimes in only one, sometimes in both nodes and was often fairly pronounced. A somewhat similar condition was observed in completely untreated rats, but on the whole the coloration was more intense in the d.b.a.-treated group. In histological features these nodes were comparable with those from other regions, modified by the action of d.b.a. Thus, red blood corpuscles were present in the usually wide lymph spaces, often in great density; while many free macrophages and ordinary reticular cells containing an appreciable number of corpuscles as well as greenish brown pigment, proved by the iron test to be 'haemosiderin', were observed in the lymph spaces and the lymphatic tissue. The presence of similar characteristics in the renal nodes of normal rats has been described in detail by previous investigators (Vincent & Harrison, 1897; Drummond, 1900; Lewis, 1903; Macmillan, 1928; and more recently Selye & Schenker, 1939). It is to be noted that the last mentioned authors succeeded in reverting such nodes to the ordinary lymph-node type by homolateral nephrectomy.

Finally, the mesenteric nodes require special comment. It will be seen from Pl. 1 that a certain number of them showed a moderate discoloration in some of the control animals. The colour was not red or brownish red, however, but a pure brown tone varying somewhat in intensity and, as a rule, involving one surface of the node. Microscopical examination, so far, has revealed that the brown tone was due to the presence in the reticular cells of a pigment of corresponding colour. It is possible that the pigment was identical with 'haemosiderin', but no attempt was made to verify this by means of the iron test. Occasionally, mesenteric nodes were found having a greenish tint. This disappeared on fixation in formol-saline and was probably due to the presence of bile.

DISCUSSION

From the whole of our results it is evident that the continuous administration of 1:2:5:6-dibenzanthracene causes a pronounced alteration in the lymphatic system of the rat: ordinary lymph-nodes are transformed gradually into haemolymph-nodes. While, under normal conditions, haemolymph-nodes were observed in the renal region only, in animals treated with d.b.a. they occurred in all the regions where lymph-nodes are situated. Thus, similar changes were produced by carcinogenic tar and by this pure carcinogenic hydrocarbon. Further investigations are in progress as to the action of two other carcinogenic hydrocarbons, 3:4-benzpyrene and methylcholanthrene. Preliminary results demonstrate that either of these compounds can induce haemolymph-nodes in the rat, similar to those formed after treatment with d.b.a.

It is of interest that Clarkson, Mayneord & Parsons (1938) described lymph-nodes with similar changes in rats which had been irradiated with X-rays, and that Selye & Foglia (1939) made similar observations on rats a few hours after exposure to various noxious agents, especially when the animals had previously received intravenous injections of large amounts of saline. The former authors also studied the action of a water-soluble derivative of d.b.a. on mice. Parsons (1938) and Parsons & Warren (1941) continued these investigations and extended them to various ordinary carcinogenic hydrocarbons as well as water-soluble derivatives. The lymph-nodes of mice so treated—particularly after prolonged administration—showed histological changes not unlike those found in the lymph-nodes of our injected rats. The main features as stated by these workers were: widening of the lymph spaces and diminution of the lymphatic tissue, and a marked increase in the number of reticular cells and free macrophages present in the lymph spaces, both kinds of cells carrying haemosiderin pigment in varying quantities, demonstrable by the iron test. With regard to the latter feature, Warren (1939) showed that the total iron content of such nodes increased gradually in the course of treatment. It appears, however, that as a rule very few or no red blood corpuscles were observed in the lymph spaces of these nodes, and that corpuscles were rarely noticed within the pigment-carrying cells. Accordingly, the surface of the nodes showed a red discoloration only in exceptional cases. Recently, Hoch-Ligeti (1941) has studied in addition the effect of d.b.a. in colloidal solution on the lymph-nodes of mice, employing the method of biopsy in order to examine different nodes of the same animal at different stages of treatment. The changes described were essentially the same as those observed by the above investigators; no statement was made concerning the presence of any extravascular red blood corpuscles. The rare occurrence of true haemolymphatic changes in the lymph-nodes of the treated mice might be due to special experimental conditions; but it is possible that it indicates a genuine difference between the mouse and rat in their reaction to carcinogenic hydrocarbons

While there seems to be certainty regarding the fate of the red blood corpuscles in the haemolymph-nodes, their origin is by no means clear and constitutes in itself an important problem. It is possible that new corpuscles were formed in these nodes, although little evidence is so far available to support this view. Moreover, on this assumption it would be difficult to understand the biological significance of the subsequent corpuscle destruction. It appears to be more likely, therefore, that the corpuscles entered the nodes from outside. That the corpuscles could have passed over directly from the blood stream is inconsistent with the fact that usually very few or no corpuscles were noticed in the immediate vicinity of the small blood vessels and capillaries, and that the lymphatic tissue in general—the seat of most of these vascular channels—always contained many less corpuscles than the sinus. There is, however, the possibility that the corpuscles reached the nodes by way of the lymphatic vessels, a possibility which has been previously considered by v. Schumacher (1912) in discussing the origin of the spontaneous haemolymph-nodes of the sheep. Accordingly, it should be assumed that throughout the whole body of the d.b.a.-treated animal there occurs a slight but continuous filtration of red blood corpuscles from the blood capillaries into the surrounding tissues. These extravascular corpuscles are then received by the peripheral lymphatics and carried along them to the regional lymph-nodes.* This view agrees with the result of Yoffey & Drinker (1939) that even in normal animals (dogs and cats) some red blood cor-

* A negligible number of corpuscles may, of course, enter the nodes directly from their own blood capillaries.

puscles are constantly entering the peripheral lymphatics. The suggested mechanism presupposes, therefore, that the hydrocarbon (or possibly a metabolic derivative) does not act directly upon the lymph-nodes. Either it would increase the permeability of the blood capillaries for red blood corpuscles, or it would alter the corpuscles themselves in a manner enabling them to penetrate more easily through the capillary wall. Special investigations will be required to find out the sequence of processes leading to the appearance of red blood corpuscles in the lymph-nodes of the d.b.a.-treated rats.

APPENDIX: THE EFFECT OF ANTHRACENE

It was of interest to compare the effect of 1:2:5:6-dibenzanthracene with that of anthracene, a related but non-carcinogenic hydrocarbon. A series of tests dealing with the action of anthracene was therefore appended to each of the four experiments described.

Rats of similar weights, and equal in number to those employed in the corresponding d.b.a. and control series, were injected with a colloidal solution of the hydrocarbon prepared similarly to that of d.b.a., the concentration being likewise 0·001 mole. per litre (i.e. 17·8 mg./100·0 c.c.). The injections, carried out in the same way as described for d.b.a., did not impair the general health of the animals and, moreover, did not inhibit the normal increase of their body-weight. Each animal was killed on the same day as a control and a d.b.a.-treated animal, and the lymph-nodes were examined in a similar manner.

As a rule, the lymph-nodes showed the same colour, and accordingly a similar histological structure, as those of control animals. Sometimes a slightly reddish discoloration was observed, involving part of the surface. Such nodes showed histologically a small number of extravascular red blood corpuscles, located mainly in the lymph spaces, while occasionally some pigment-carrying cells were noticed. In exceptional cases, however, more pronounced changes were found. Thus in one animal (fifteen injections) a submaxillary node of dark red colour was observed, in another (twenty injections) a markedly red cervical node. In a third animal (seventeen injections) a number of mesenteric nodes with an intense brownish red discoloration was found. The histological changes present in these nodes were similar to those present in deeply discoloured nodes from d.b.a.-treated animals, numerous extravascular red blood corpuscles as well as many pigment, etc. carrying cells being observed. Notwithstanding these few greatly changed nodes, the effect of anthracene was, in general, strikingly less than that of 1:2:5:6-dibenzanthracene.

The specimen of anthracene used in these investigations was of light yellow colour. In accordance with Winterstein, Schön & Vetter (1934) it should be assumed that this colour was due to a trace of naphthacene and that the specimen contained in addition a small amount of carbazole. While, so far, no carcinogenic action has been ascribed to them, it is conceivable that these impurities, and not anthracene itself, were responsible for the pronounced effect upon a few lymph-nodes, and possibly also for the lesser changes noticed in other instances. Further investigations on the effect of purified anthracene and purified phenanthrene (which is also non-carcinogenic) are in progress, and so far it appears that very little or no changes can be induced in the lymph-nodes of rats treated with them.

SUMMARY

The effect of 1:2:5:6-dibenzanthracene on the lymph-nodes of the rat has been investigated. The hydrocarbon was administered subcutaneously in the form of an aqueous colloidal solution, injected over a period of several weeks.

It was found that many of the lymph-nodes of the animals thus treated were transformed into more or less pronounced haemolymph-nodes, characterized macroscopically by a red or brownish red discoloration of varying extent and degree. On microscopical examination the following principal changes were observed in these nodes: (1) widening of the lymph spaces and corresponding diminution of the lymphatic tissue; (2) presence in the lymph spaces of red blood corpuscles, frequently in considerable numbers; (3) increase in the number of free macrophages which were loaded with ingested corpuscles and haemosiderin pigment. The implications of these findings are discussed.

Similar experiments were carried out with anthracene, and it was found that this hydrocarbon on the whole was much less effective than 1:2:5:6-dibenzanthracene.

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Experiment
Rat no.
Number of injections
At death
Days after first injection
Days after last injection
Submaxillary nodes
Cervical nodes, l.
Cervica nodes, r.
Axillary nodes, l.
Axillary nodes, r.
Inguinal nodes, l.
Inguinal nodes, r.
Thoracic nodes
Mesenteric nodes
Lumbar nodes
I
1 15 21 4
2 19 27 3
3 20 28 1
4 22 30 1
II
1 15 21 1
2 17 23 1
3 18 24 1
4 20 28 1
5 20 28 1
6 22 30 1
7 22 30 1
8 22 44 15
III
1 15 17 2
2 15 20 5
3 20 28 1
4 20 33 6
5 20 35 8
6 20 39 12
7 20 41 14
8 20 41 14

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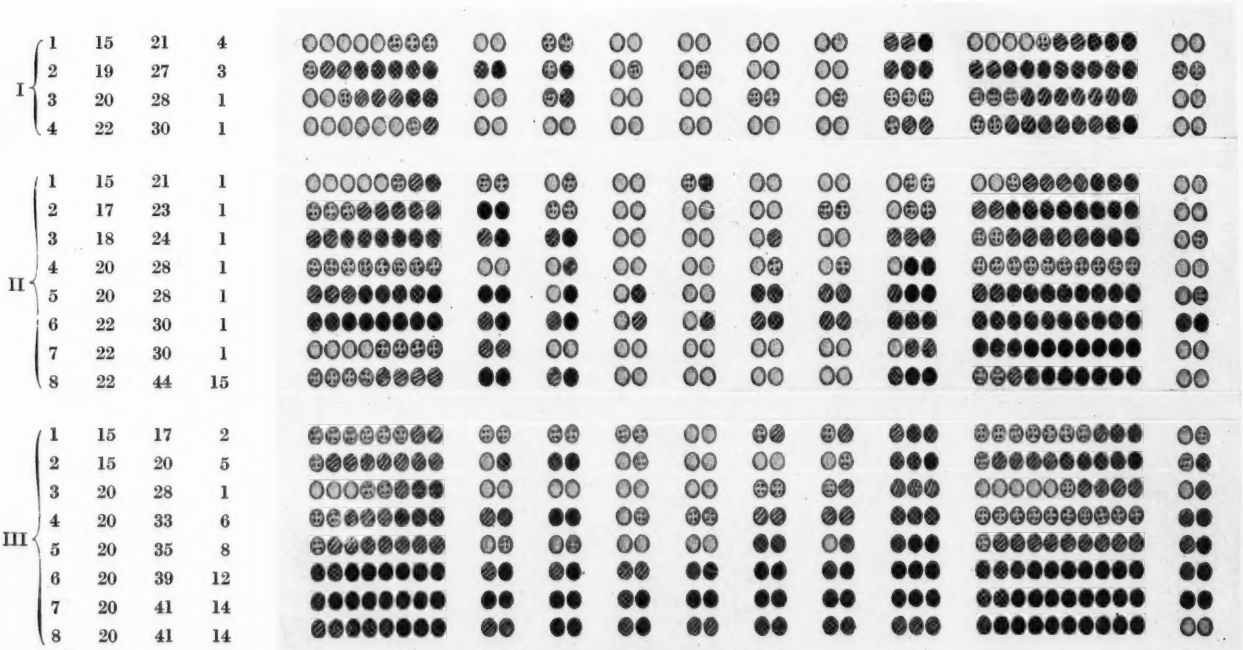
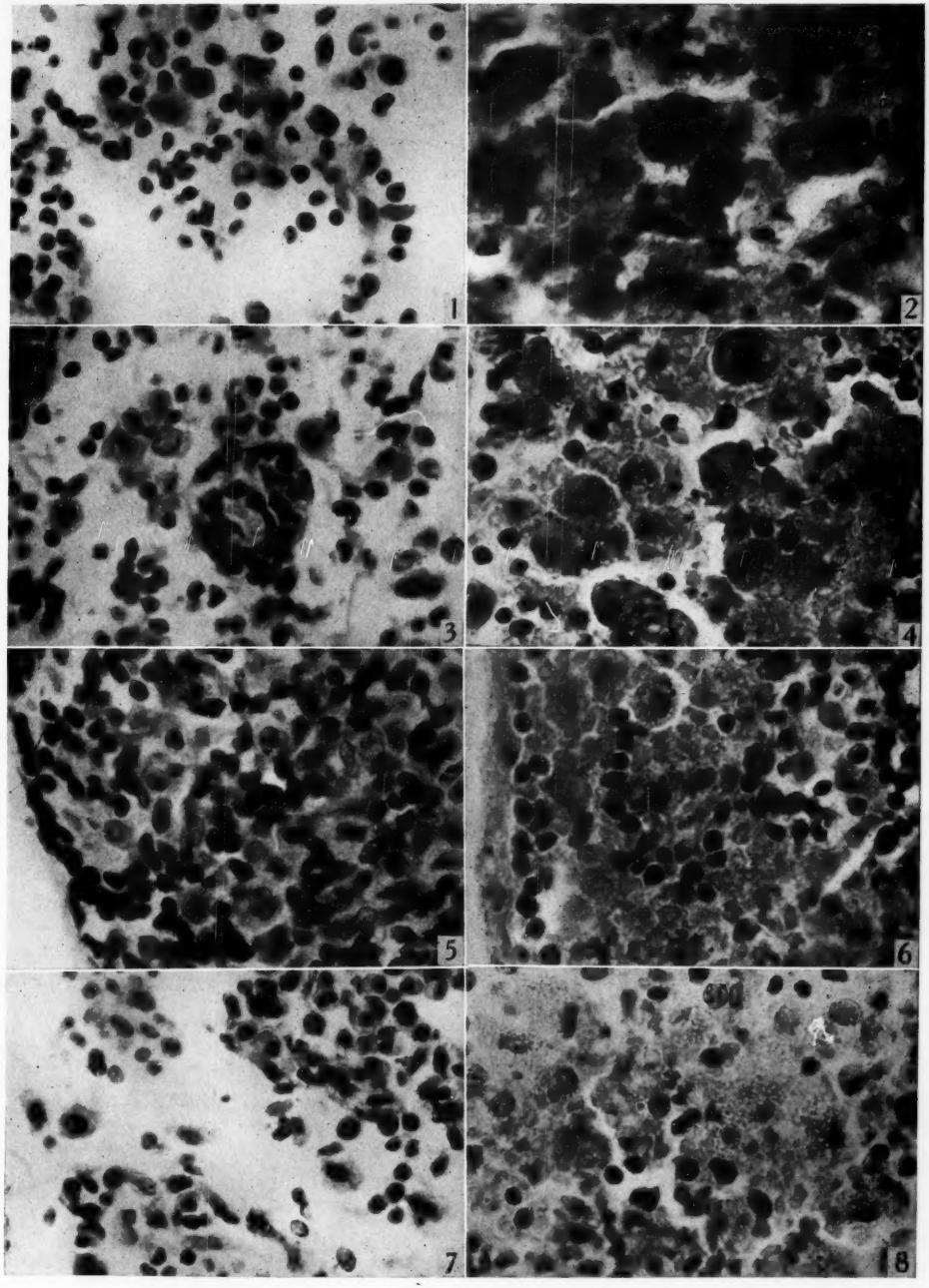


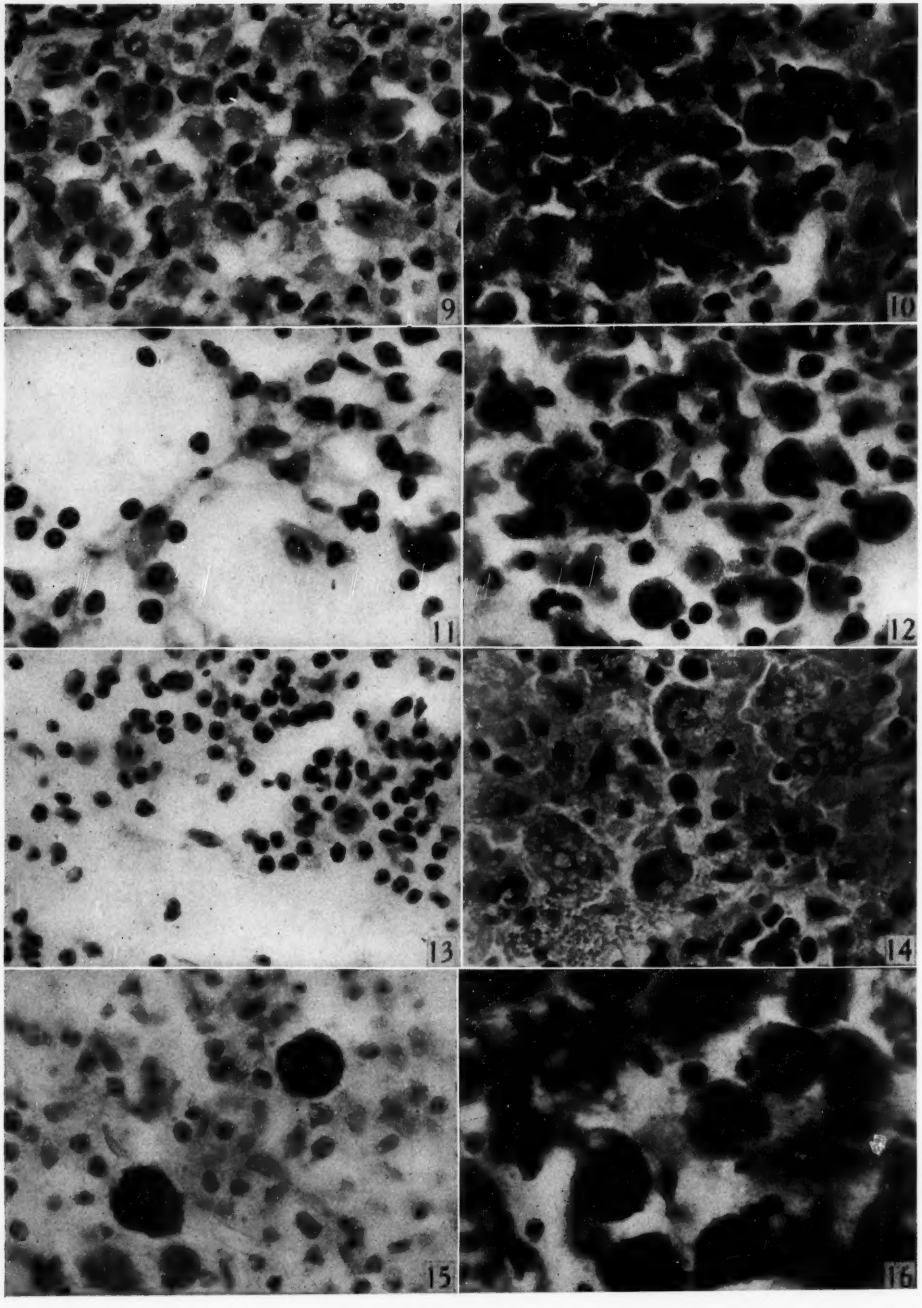
LASNITZKI AND WOODHOUSE—THE EFFECT OF 1:2:5:6-DIBENZANTHRACENE ON THE LYMPH-NODES OF THE RAT

Plate 1

MICH.



LASNITZKI AND WOODHOUSE—THE EFFECT OF 1:2:5:6-DIBENZANTHRACENE ON THE LYMPH-NODES OF THE RAT



LASNITZKI AND WOODHOUSE—THE EFFECT OF 1:2:5:6-DIBENZANTHRACENE ON THE LYMPH-NODES OF THE RAT

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EXPLANATION OF PLATES

The sections shown in figs 1–14 were stained with haematoxylin and eosin. Those shown in figs. 15 and 16 were treated for iron precipitation. For further details, see text.

Plate 1

The appearance of the lymph-nodes of rats after injection of control solution (upper field) and d.b.a. solution (lower field).

Plate 2

Fig. 1. Submaxillary node, control rat.
Fig. 2. Submaxillary node, d.b.a.-treated rat.
Fig. 3. Cervical node (r.), control rat.
Fig. 4. Cervical node (r.), d.b.a.-treated rat.
Fig. 5. Cervical node (r.), control rat.
Fig. 6. Cervical node (r.), d.b.a.-treated rat.
Fig. 7. Thoracic node, control rat.
Fig. 8. Thoracic node, d.b.a.-treated rat.

Plate 3

Fig. 9. Mesenteric node, control rat.
Fig. 10. Mesenteric node, d.b.a.-treated rat.
Fig. 11. Mesenteric node, control rat.
Fig. 12. Mesenteric node, d.b.a.-treated rat.
Fig. 13. Lumbar node, control rat.
Fig. 14. Lumbar node, d.b.a.-treated rat.
Fig. 15. Cervical node (l.), d.b.a.-treated rat.
Fig. 16. Lumbar node, d.b.a.-treated rat.

POST-NATAL GROWTH CHANGES IN THE HUMAN PROSTATE

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From the Department of Anatomy, University of Oxford

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I. INTRODUCTION

In spite of the very extensive researches which have been carried out upon the subject of benign enlargement of the prostate, it remains a condition which is very prevalent among old men, causing them great discomfort and inconvenience, for the relief of which recourse has still to be made to surgery in the majority of cases. It is true that such treatment can lay claim to many successes, but it is, nevertheless, by no means devoid of dangers, and from the standpoint of rational medicine, it must be considered as a measure which is adopted because no more scientific method of treating this malady is known.

The literature contains relatively few accounts of the normal growth of the prostate, and since a sound knowledge of normal growth is an indispensable basis for the elucidation of pathological growth, it was considered that an investigation of normal growth would be a not unprofitable prelude to the study of benign prostatic enlargement, the results of which are to be published elsewhere. In the account which follows, there are included histological observations on all the principal systems of the prostate—glands, collecting ducts, uterus masculinus, ejaculatory ducts and urethral mucosal glands—at the various stages of its post-natal development. These comprise the condition at birth, before puberty, at puberty, and in the adult.

Growth curves, relating the variation of prostatic volume with age, have also been constructed.

Material and methods. For the greater part of the work, material has consisted of prostates obtained at routine autopsy from the London Hospital, the Radcliffe Infirmary, Oxford, and Birmingham. These prostates were fixed in formalin or alcoholic Bouin's fluid, some of them having previously been bisected transversely or slit along the anterior commissure. A number of operative specimens of enlarged prostates were also available. The volumes of most of the prostates were measured by the method of water displacement; before measurement, which was always carried out with the fixed specimen, all the surrounding connective tissue, bladder, seminal vesicles, etc., were trimmed away so as to give an accurate measure of the size of the prostate itself.

A total of 113 prostates were prepared for histological examination; most of these were cut into about five transverse slices and a few sections taken from each; some were sectioned serially throughout. The sections were variously stained in haematoxylin and eosin, Masson's trichrome stain, orcein and Van Gieson, and Weigert's elastin stain.

For the construction of the growth curves, 192 prostates of all ages were used. Details of the prostate material used are shown in Table 1.

Table 1. *Details of human prostate material used*

Age group	All specimens	Prepared for histology	Volume known
At birth	8	8	5
Up to 5	7	3	5
6–10	6	4	6
10–15	4	3	4
16–20	9	4	9
21–25	10	3	10
26–30	11	5	11
31–35	16	3	16
36–40	15	7	15
41–45	12	5	12
46–50	16	7	16
51–55	18	10	15
56–60	18	8	18
61–65	26	11	24
66–70	10	5	9
71–75	11	4	11
76–80	5	1	5
Over 80	1	—	1
Age unknown	35	22	—
Totals	238	113	192

II. THE PREPUBERTAL PROSTATE

Embryology

As this work is concerned with the growth changes occurring in the prostate after birth, it will not be expedient to enter into any detailed discussion of the embryology of the organ. The most complete account of this has been given by Lowsley (1912), to whose paper the reader is referred for further details; it will suffice here to mention that Lowsley described the prostate gland as originating from five independent groups of tubules which begin to develop in the wall of the proximal part of the urethra at about the twelfth week of intra-uterine life. These groups form the median or prespermatic lobe, a pair of lateral lobes, an anterior lobe and a posterior lobe.

The prostate at birth

At birth, the prostate is composed chiefly of connective tissue with a relatively small proportion of intermixed smooth muscle. Embedded within the stroma so formed is the duct system radiating outwards from its origin in the urethra. At this stage, as in all succeeding ones up to puberty, the ducts end blindly near the periphery and the alveoli of the adult prostate are represented only by end-buds. The form of the urethra is the same as in the adult prostate and from it, in addition to the ducts already mentioned, there leads the uterus masculinus, the opening of which is situated in the median plane on the verumontanum, usually immediately cranial to the openings of the ejaculatory ducts, although it is not altogether uncommon to find the order reversed.

Prostatic ducts. The prostatic ducts open into the urethra chiefly in the distal half to two-thirds of its prostatic part, but excluding its distal tenth or so. The openings are grouped around the prostatic sinuses, a variable proportion opening into the floor of the sinuses, with the remainder opening on the lateral parts of the verumontanum or on the lateral walls of the urethra. Ducts of the anterior lobe may be present, when they usually lead straight back into the ventral wall of the urethra; they exist only in the caudal third of the organ.

Typically, the ducts are composed of cubical or low columnar cells with large, centrally placed nuclei. Two layers of cells may be regarded as the basic arrangement but in many parts of the prostate this number is exceeded to a varying extent. In places the ducts may appear as solid cords of cells; whether these are the primary cords which have not yet become canalized, or whether they are ducts whose lumina have secondarily become obliterated is a matter for conjecture. The fact that in the prostate at birth many of the ducts are patent in their peripheral parts, while the solid cords are found nearer the centre, is evidence in favour of the view that the solid cords are formed secondarily rather than that they are persistent uncanalized ducts. Here and there in these cords can be found the appearance of pseudo-acinar formation (Pl. 1, figs. 1, 2). The same appearance is seen in the thickened hyperplastic walls of patent ducts, and is similar to that found in certain tumours, such as prostatic adenocarcinoma and ovarian papillary cystadenoma, and also in chronic cystic mastitis. It is also strictly comparable with that found by Zuckerman & Sandys (1939) in the Hanuman langur (*Presbytis entellus*) subjected to oestrogenic stimulation, and, as those authors suggest, may well be part of a process of budding off of new ducts. In a rather small proportion of cases, these pseudo-acini contain eosinophilic colloidal material which may represent products of cell breakdown.

In addition to the hyperplasia of the epithelium of the ducts mentioned above, squamous metaplasia is frequently found. It most commonly affects ducts in the region of the prespermatic lobe and may lead to their complete blockage (Pl. 1, fig. 4). Here again, pseudo-acinar formation may occur. Metaplasia of the duct epithelium is not found in the periphery of the prostate.

End-buds. In general, the epithelium of the end-buds resembles that of the ducts; many of the end-buds possess no lumen, though this is not true for all (Pl. 1, fig. 5). Occasionally, end-buds are found with a secretory epithelium of tall columnar cells with basal nuclei, the type, in fact, being that of the active adult prostatic alveoli (Pl. 1, fig. 6). Moore (1936*a*) has drawn attention to this phenomenon and stresses its essentially focal nature, which he regards as expression of subtotal stimulation; the probable nature of the stimulus will be discussed later (p. 132).

Urethra. The epithelium lining the urethra is fundamentally of the transitional type, there being a varying number of somewhat irregularly disposed layers of cells (the number may reach 10), of which those next the lumen are cuboidal in shape, being slightly flattened tangentially, while the more deeply lying cells are cubical or of very short columnar type. In general, the above description is true for both the anterior and the posterior walls of the urethra, but very commonly, the epithelium overlying the verumontanum* shows areas of hyperplasia, the number of layers being considerably increased, while the luminal layers show squamous metaplasia, the cells being pale staining

* The posterior wall of the urethra bulges forwards as the crista urethralis; this bulge is most prominent in the region of the openings of the ejaculatory ducts and uterus masculinus (verumontanum or colliculus seminalis). The portions of the crista above and below the verumontanum are sometimes referred to as the superior and inferior cristae respectively.

and undergoing keratinization (Pl. 2, fig. 8). The extent of this metaplasia is variable; occasionally it is not found at all, or it may occur only in a few isolated pockets. On the other hand, it may extend throughout the crista urethralis and reach onto the anterior walls of the urethra, these extensions usually being more or less focal.

Small crypt-like infoldings of the epithelium are generally found, being most frequent in the region of the prostatic sinuses; they probably represent the forerunners of the mucosal glands.

Uterus masculinus. The uterus masculinus is a conspicuous feature of the new-born prostate, often being very large and distended (Pl. 1, fig. 7; Pl. 2, fig. 10). Frequently it extends cranially almost to the entrance of the ejaculatory ducts into the posterior surface of the prostate. It lies, together with the ejaculatory ducts, in a stroma which is somewhat less dense (possibly because it is slightly oedematous) than that of the remainder of the prostate and which is practically devoid of smooth muscle fibres. This stroma is usually clearly demarcated from the remainder of the prostate by a concentric condensation of smooth muscle and connective tissue forming a capsule.

The form of the uterus masculinus is variable, and, in this connexion, Vintemberger (1926) described two main types: the diverticular and the glandular type, with intermediate forms. He found that the differentiation could be traced back even to the foetal stage, although in its early development the uterus masculinus consists merely of solid cords of cells. As seen in the new-born, the uterus masculinus is most commonly represented by a distended sac, from which a few duct-like diverticula may extend a short distance into the stroma; they have not been observed to penetrate the capsule. This is the diverticular type of Vintemberger. The glandular type of utricle, less commonly seen, consists at birth of an irregular series of cords of cells passing in all directions from a main lumen, which may be very small, and which has sometimes been observed to open into the urethra by two mouths. Occasionally, the utricle is T-shaped in cross section, its main lumen not being distended; this form is reminiscent of that found in many species of monkey.

Fundamentally, its epithelium consists of small cuboidal cells, but it is usual to find extensive hyperplasia with squamous metaplasia of the same type as previously described for the prostatic ducts and verumontanum. Hyperplasia and metaplasia are found in the diverticula also. The lumen of the uterus masculinus is frequently occupied by a mass of desquamated débris.

Ejaculatory ducts. The ejaculatory ducts are lined by an epithelium consisting of two layers of cells, those next the lumen being short columnar while those of the basal layer are cubical in shape. The nuclei are large and occupy the greater part of the cells. Occasionally, the epithelium is hyperplastic near the urethra.

The foregoing account shows that hyperplasia and squamous metaplasia of the epithelium of the ducts, verumontanum and uterus masculinus is a conspicuous feature of the new-born prostate and it may be mentioned that it was first reported by Aschoff (1894) and confirmed by Schlacta (1904). It is of precisely the same nature as that observed in the prostate of the rhesus monkey (*Macaca mulatta*) subjected to oestrogenic stimulation (van Wagenen, 1935; Parkes & Zuckerman, 1935; Zuckerman, 1938) and there seems little doubt that it results from the action of maternal oestrogens circulating in the foetal blood. This view was taken by Halban (1904, 1905) on purely theoretical grounds, and has recently been confirmed by Sharpey-Schafer & Zuckerman (1941) who studied the effects of oestrogens on the prostates of babies. Unlike the rhesus prostate subjected to prolonged oestrogenic stimulation, the ejaculatory ducts of the new-born prostate do not, as a rule, show any hyperplasia at their terminations and, following Zuckerman's suggestion (1936 *a*, *b*), it is therefore to be presumed that no invasion and replacement of Wolffian epithelium by urogenital sinus epithelium normally occurs in man.

The occasional occurrence of apparently actively secreting prostatic glandular epithelium would appear to require for its explanation a stimulation by male hormone and this is the view to which Moore (1936*a*) subscribes, its essentially focal distribution being interpreted as the result of subtotal stimulation. The presence of small amounts of male hormone can be ascribed to the action of maternal anterior pituitary gonadotropic hormone stimulating the foetal testis with a consequent elaboration of the androgen.

Urinary retention and hydronephrosis in new-born babies have been ascribed to prostatic obstruction resulting from oestrogenic stimulation; it seems doubtful, however, whether this explanation is correct. Although showing unmistakable signs of oestrogenic stimulation, the new-born prostate is seldom enlarged to any extent. In one personally investigated case of retention with hydronephrosis the prostate was definitely of normal size (volume 1·25 c.c.). There was no difficulty in passing a catheter, and the verumontanum, though prominent, was in no way pathological; hyperplasia and squamous metaplasia were not excessive, nor could enlargement of the uterus masculinus be considered as sufficient to have caused obstruction. On the other hand, there existed at the internal urethral meatus a pair of thin, valvular mucosal folds on either side, so shaped and disposed as to

prevent the passage of urine, while offering little obstruction to the passage of a catheter (Pl. 2, fig. 9). In this case, doubtless, the obstruction was due to these mucosal valves, an account of whose congenital occurrence has been given by Young & McKay (1929), and it is probable that a similar state of affairs existed in the other cases of urinary obstruction in the new-born which have been reported.

The post-natal prepubertal prostate

Within 6 or 7 weeks of birth, the specific indications of oestrogenic stimulation disappear from the prostate: the metaplasia of the epithelium of the verumontanum, terminal parts of the prostatic ducts and uterus masculinus is no longer evident, while the distension of the last subsides so that it assumes the shape of a sagittal slit, not infrequently with a T-shaped extension anteriorly. In this condition the prostate continues up to about the ninth year with very little increase in size or differentiation.

Prostatic ducts. The most conspicuous feature of the prepubertal prostate is the extensive hyperplasia of the duct epithelium. This frequently proceeds to complete blockage of the lumen. Subsequently, pseudo-acinar formation occurs, often associated with lateral proliferations from the ducts which represent a process of development of side branches whereby the duct system undergoes elaboration. Colloid may be found in these new lumina, and might be derived from the products of disintegrated cells, although secretion cannot be excluded, and, in fact, would seem to be the most likely explanation of the large amounts of colloid seen in some of the older prepubertal prostates (see p. 133). The process of hyperplasia and pseudo-acinar formation is found chiefly in the central parts of the prostate; near the periphery, hyperplasia of the epithelium, though found, is not extensive.

End-buds. The end-buds of the prostatic ducts tend to become more patent with advance in age, though they remain small in size and their epithelium is not of the true secretory type. This epithelium consists of a single layer of cells, except in the poorly differentiated end-buds where there may be two or three layers; the cells are very short columnar with fairly large nuclei which can already be seen assuming a basal position similar to that found in the fully differentiated alveolar epithelium.

Urethra. The general character of the urethral mucosa remains similar to that already described, except, of course, that metaplasia is no longer found. As a rule, some degree of hyperplasia is present and the crypt-like infoldings tend to increase in number. Often, in the walls of these, and in the unfolded mucosa besides, foci of pseudo-acinar formation are to be found, leading, it would seem, to the development of the urethral mucosal glands (Pl. 2, fig. 11). This occurrence in the hyperplastic urethral mucosa may be regarded as additional evidence for the correctness of the same interpretation of the comparable appearance in the hyperplastic duct epithelium, where, as was mentioned, some doubt could exist concerning the possibility that the foci were really the remains of a persistent lumen rather than a newly formed one. Where the foci of pseudo-acinar formation are found in the urethral mucosa there is little likelihood of confusing them with persistent duct lumina.

Uterus masculinus. The uterus masculinus is lined by an epithelium consisting of two or three layers of cuboidal cells with fairly large nuclei. In most cases, numerous duct-like diverticula are present and these show extensive hyperplasia, with foci of pseudo-acinar formation, eosinophilic colloid being found in some of the lumina. A characteristically complex glandular type of uterus masculinus in a five-year-old prostate is shown in Pl. 2, fig. 12. The stroma is still somewhat less dense than that of the remainder of the prostate, but now some smooth muscle fibres are to be found. The surrounding capsule is usually distinct and contains smooth muscle fibres.

Ejaculatory ducts. The ejaculatory ducts call for no comment, answering to the description already given.

Anterior lobe. Occasionally, ducts of the anterior lobe, distinguishable from anterior extensions of the lateral lobes, are found. They drain backwards into the ventral urethral wall and show features similar to those of the lateral lobes.

Between ages of about 9 years and puberty, cyst-like dilatations of the prostatic ducts are very commonly found, the lumina being filled with eosinophilic colloid (Pl. 3, fig. 14). Although hyperplasia is frequently intense, it seems unlikely that cell breakdown could account for the quantities of colloid found and it may therefore be presumed that the epithelial cells possess some secretory ability, although even at this stage, the occurrence of epithelium of the true secretory type is rare.

III. CHANGES AT PUBERTY

The transformation of the prepubertal prostate into the adult type of organ takes place during a relatively very short period of time, probably a matter of 6 months to 1 year, and because of this, it is naturally a difficult matter to obtain prostates in the intermediate stages. Thus, whereas a specimen 13 years of age may show only slight signs of pubertal changes, another of 14 years may be in the full adult state (see examples, p. 134 et seq.). The increase in size of the prostate at puberty is

very rapid, especially when contrasted with its rate of growth before puberty. From birth to 13 years the average volume increases from about 1 to 4 c.c., while between the thirteenth and fourteenth years the volume rises to about 10 c.c. (see Text-figs. 1, 2). This enlargement is due almost entirely to the development of the alveolar system.

The earliest changes seen consist of enlargement of the lumina of the end-buds of the prostatic ducts and of the thinning out of their epithelium to a single cell layer. The cells become elongated radially and their nuclei assume a basal disposition. In most cases the cells remain small, though here and there they attain proportions nearer to those of the adult alveoli. Colloidal material may be found, but it is more common in the distended ducts already mentioned. Many of the ducts remain hyperplastic, with foci of pseudo-acinar formation. The epithelium of the urethra also is hyperplastic and, especially over the verumontanum, tends to be folded in complex fashion, with numerous areas of pseudo-acinar formation.

Alveoli. The further development of the alveolar system consists in the extension to all the end-buds of the changes already described, and their subsequent elaboration to attain the typically adult condition. In many cases this process does not reach completion for some years, so that it is not uncommon to find groups of juvenile end-buds in otherwise fully differentiated prostates up to the age of 20 years or more (Pl. 2, fig. 13). The alveoli are somewhat distended and the epithelium shows numerous peninsular extensions into the lumina. The larger of these extensions are supported on delicate stromal processes but in the smaller extensions the epithelium appears simply to be bulged into the lumen with only the basement membrane intervening between the two layers of epithelial cells. Typically, the cells are tall columnar with lightly staining basophilic cytoplasm and fairly large basally disposed nuclei. Owing to the elaborate nature of the epithelium, any given section will pass through the cells in various planes and so may give rise to the appearance in some places of several layers of cells of varying shape, but careful examination is usually sufficient to indicate the cause of this. The luminal borders of the cells are often indefinite as a result of the adherence to them of what are presumably secretory products. The stroma is condensed around each alveolus and in most cases contains a fairly high proportion of smooth muscle fibres.

Prostatic ducts. Characteristic changes are undergone by the prostatic ducts, in which hyperplasia of the epithelium is still to be found but now the foci of pseudo-acinar formation coincide with the site of alveolus development, the cells surrounding the new lumina elongating and assuming the same characteristics as those of the prostatic alveoli themselves (Pl. 3, fig. 16). In this way the ducts themselves come to be part of the alveolar system and the secretory epithelium may extend right up to their openings into the urethra (Pl. 4, fig. 20). A variable number of ducts, however, generally retain a non-secreting type of epithelium consisting of the usual cuboidal cells.

Uterus masculinus. Changes occur in the uterus masculinus also, the diverticular epithelium tending to become of secretory type and hyperplasia becoming reduced in intensity. As a rule, however, it is not until two or three years later that definitely secretory alveoli are to be found in the uterus masculinus.

The changes in the prostate at puberty can more adequately be illustrated by reference to brief descriptions of some typical prostates taken just before and during puberty. Three such examples are given below:

H.P. 22—*age* 10 *years. Glandular system.* This is undifferentiated and consists of a proliferating series of ducts radiating posteriorly and laterally with some anterior extension of the most laterally disposed ducts. Most of the ducts are canalized. The epithelium, while consisting typically of about two layers, is in many places hyperplastic, especially near the urethra. The anterior extensions of the lateral lobes consist almost entirely of solid cords of cells.

There is a well-developed anterior lobe, the duct epithelium showing extensive hyperplasia. These ducts open into the anterior wall of the urethra.

Urethra. Numerous mucosal glands are present in the anterior wall, their epithelium closely resembling that of the urethra itself. This last consists mostly of from four to six layers of tightly packed cells with intensely basophilic nuclei.

Uterus masculinus. Cranially, this consists of a horseshoe-shaped main lumen, the central part of which is canalized while the postero-lateral limbs are solid cords of cells. There are numerous diverticula, mostly composed of solid cellular cords.

More caudally, there is a central T-shaped lumen from which solid cords extend anteriorly and, to a less extent, laterally along the crista lateral to the ejaculatory ducts; others extend posterior to the ejaculatory ducts. Circularly disposed muscle fibres demarcate the entire utricular system fairly clearly but do not enclose it completely.

H.P. 97—*age* 37 *years. Glandular system.* This is mainly similar to that described above. There is an anterior lobe in the caudal third of the prostate. Though the ducts themselves are mostly canalized, end- and side-knobs are generally solid. Only a few of the ducts near the periphery are distended but those nearer the urethra, i.e. in the utricular bed, are very distended and filled with eosinophilic colloid (Pl. 3, figs. 14, 15).

Epithelial hyperplasia is practically restricted to ducts in the utricular bed and the anterior lobe.

Urethra. The epithelium consists of many layers of cells

resembling the epithelial cells of the rest of the prostate. Mucosal glands are richly developed.

Uterus masculinus. The main lumen is a distended irregular cyst lying anterior to and between the ejaculatory ducts; it is lined with from four to ten layers of tightly packed cells with somewhat irregular, relatively large nuclei. Densely solid cords of cells radiate anteriorly and posteriorly. Differentiation of definitely utricular extensions from epithelial elements derived from the prostatic duct system proper is very difficult (Pl. 3, fig. 15).

H.P. 49—*age* 15 *years.* For the most part the prostatic ducts and acini are fully differentiated, the latter being lined by a single layer of columnar secretory cells with small basal nuclei. In most alveoli the epithelium is folded and projects as fringe-like processes into the main lumen. In many cases these processes are supported on a central stromal projection. Many of the acini and ducts are distended with secretion. Within the posterior and lateral parts of the gland none of the acini appear to be undifferentiated; in the anterior extensions of the lateral lobes, however, the ducts and alveoli are not differentiated and have the characteristics typical of immature prostates. Hyperplasia of the epithelium is also found in the ducts of the prespermatic region.

Urethra. Anteriorly the urethral epithelium is composed of spindle cells showing slight hyperplasia; there are a few shallow crypts. Posteriorly it consists chiefly of columnar cells, with moderately deep crypts in the more cranial levels. There are numerous mucosal glands on the crista, where the epithelium consists of from four to ten layers of cells.

Uterus masculinus. This consists of a short sagittal diverticulum of the crista directed backwards and slightly to one side. A few crypt-like out-pouchings extend a short distance from the main lumen. Hyperplasia of the epithelium is widespread, being particularly conspicuous in the crypts. Mucinous degeneration is found here and there posteriorly.

The opening of the uterus masculinus extends further caudally than do those of the ejaculatory ducts. The stroma surrounding the organ is slightly, but not at all clearly, differentiated from the remainder of the prostate.

With regard to the factors underlying the changes at puberty, there can be no doubt that they are due to the action of the male hormone, whose secretion by the testis in effective amounts commences at this time. The fact that castration before puberty leads to failure of development of the prostate, along with the other accessory sexual organs, has long been known. John Hunter (1786) pointed out that 'the prostate and Cowper's glands, and those of the uretha, which, in the perfect male are soft and bulky, with a secretion salt to the taste, in the castrated animal are small, flabby, tough and ligamentous and have little secretion'. Berthold (1849) gave the first scientific proof of the implication of the testis in the effects of castration, when he showed that these effects in cocks could be prevented by testicular grafts; this was the first demonstration of a specific hormonal mechanism. The most direct proof of the prostatic changes at puberty being the result of the action of the male hormone has been given by studies of the results of its administration to monkeys (Zuckerman & Parkes, 1936*b*, 1938).

There still remains the question of the causation of the hyperplasia observed in the ducts of the prepubertal prostate and for the moment it seems that stimulation by very small quantities of male hormone provides the most likely explanation, on the basis that weak androgenic stimulation may cause more or less undifferentiated growth of sensitive epithelium, while stronger stimulation results in the differentiation of secretory cells such as is known to occur. In support of this suggestion it may be noted that the prostate of the rhesus monkey subjected to weak androgenic stimulation shows some hyperplasia of the ducts resembling that found in the prepubertal human prostate. It is necessary, however, to bear in mind that oestrogens also cause hyperplasia of at least the terminal parts of the ducts, a fact which has been shown experimentally in the Hanuman langur by Zuckerman & Sandys (1939) and has been reported as occurring in the human prostate by Moore & McLellan (1938). It is also well known that oestrogens are excreted by adult males, though regarding their excretion before puberty knowledge is less certain. Nathanson, Towne & Aub (1941), however, have recently reported on the excretion of sex hormones in childhood. They find that from 3 to 7 years of age, both boys and girls excrete a small amount of oestrogens and 17-ketosteroids (androgens) in the urine, there being very little sex difference. From 8 to 11 years there is an increased excretion of these hormones, that of 17-ketosteroids increasing more rapidly in boys, that of oestrogens in girls. On the whole, therefore, the first explanation seems to be the more acceptable, though a synergic action between very small amounts of both hormones cannot be excluded.

IV. THE ADULT PROSTATE

In this section are discussed those changes which occur in the prostate after puberty. The stroma of the prepubertal prostate constitutes a fraction of the whole which greatly exceeds that contributed by the epithelial elements, but at puberty the position becomes reversed. So it remains throughout the greater part of adult life except that when senile involutional changes set in, there is a corresponding diminution in the proportion of the epithelial elements. During the second and third decades growth in the size of the whole prostate is rapid and, in general, is due chiefly to an increase in the number of glandular alveoli. Thereafter, the average size of the prostate remains fairly constant

up to the age of fifty, as will be seen in a later section. In the sixth and later decades, the size of the prostate varies in a complex fashion related to the frequent occurrence of pathological processes. These, however, will not be discussed here, for we are concerned only with what may be considered as normal growth changes, and these are most probably involutional during this latter period of life.

Lobes of the prostate. It is necessary, in the first place, to reconsider the arrangement of the glands of the prostate since differences exist in this respect between the prepubertal and the adult prostate. It will be recalled that Lowsley considered the foetal prostate to contain, at least during a certain period, five lobes, comprising two lateral lobes, an anterior, a median and a posterior lobe. Of these, the anterior lobe is generally recognized as undergoing atrophy in the majority of instances and as being represented in only a small proportion of adult prostates. The median lobe is present in most cases in the adult, although its degree of development varies very considerably in different individuals.

In a few cases no glandular tissue can be distinguished in the region of the prespermatic or median lobe, while, in yet others, such glandular tissue as may be present in this region is derived by the medial encroachment of the lateral lobes forming a prespermatic commissure. It is difficult to be certain of the exact origin of the prespermatic glandular tissue in many cases, but it seems probable that this is more often commissural than has generally been supposed. In this connexion it may be pointed out that in the prostates of several species of monkey the lateral lobes form, in the cranial parts, unmistakable pre- and retrospermatic commissures, and there seems to be good reason to suppose that a similar state of affairs exists in man.

Concerning the posterior lobe as described by Lowsley, there is considerable doubt as to whether it finds any representation in the majority of adult prostates. Certain it is that no definite fibrous lamella can be found separating the lateral lobes from glandular tissue occupying the position described by Lowsley for the posterior lobe. Strongly suggestive evidence for the non-existence of a posterior lobe in the majority of cases has been obtained by LeDuc (1939) using a technique involving the injection of prostatic ducts with Indian ink. Special efforts to inject ducts whose orifices lay in the posterior urethral wall caudal and lateral to those of the ejaculatory ducts, that is, in the area described by Lowsley as containing the orifices of the ducts of the posterior lobe, showed that of fifty ducts injected (thirty-six on the inferior crista, fourteen in the distal urethra) only four led directly posteriorly and that of these, only two corresponded in any way with Lowsley's description. These two ducts led directly up to the mid-line, posterior to the urethra and ejaculatory ducts but did not drain any tissue of the lateral lobes. The other two were such short and rudimentary ducts that no importance could be attached to them. In several specimens, however, lateral lobe ducts could be seen to form a retrospermatic commissure and this LeDuc regards as being the most usual arrangement.

Central glands. A further group of glands, of which only small mention has so far been made, is that to which the names inner, central, periurethral, accessory and submucosal have variously been given. They were first described in 1894 by Jores who located them in groups on the trigone and vesical neck, and in the urethral walls below the neck of the bladder. Subsequently, these glands were found to be sparse in early life, but to increase in number in succeeding age groups, although in some instances they are absent altogether. Several other authors have described these glands, including Albarran & Motz (1902), Loeschke (1920), Adrion (1922), Jacoby (1923), Young & Davis (1926) and Stieve (1930). Jacoby drew attention to their wide variation in size and distribution and showed that some of them might drain into ducts of other prostatic glands. Stieve was unable to discover any difference in cellular structure between the central and the outer prostatic glands.

Attention was first drawn to the central glands as a result of the paper of Motz & Perearneau (1905) wherein these authors sought to show that all hyperplasia of the prostate has its origin in these glands. Subsequently evidence has accumulated which throws doubt upon this hypothesis, and the importance of the central glands has diminished accordingly. In view of the findings of Jacoby and of Stieve mentioned above, it may be questioned whether or no any useful purpose is to be served by the continuation of the practice of describing central and outer prostatic glands.

Alveolar epithelium. The alveolar epithelium of the adult prostate shows considerable variation in different prostates and in different parts of the same prostate. As Macklin (1928) points out, there is a striking lack of harmony in the descriptions of the numerous writers regarding almost every point of discussion. In the first place, there is disagreement as to the number of layers of cells, a single layer being described by Walker (1910) for the adult rat and by Weski (1903) for the human subject. The latter author occasionally finds a second layer of flattened elements crowded between the bases of the columnar cells, and here the structure is such that the nuclei of the latter are above the level of the free border of the former, thus giving rise to the false impression of a double layered condition. A number of workers consider two layers as normal, describing a stratum of flattened or cuboidal cells underlying the lining of cylindrical cells (Langerhans, 1874; Rüdinger, 1883; Disselhorst, 1904, for the human as quoted by Petersen, 1909; Prenant &

Bouin, 1910). Personal observation leads the present writer to favour the opinion that a single layer is the normal arrangement.

Petersen (1909) described a secretory cycle for the epithelial cells and the later description of Moore (1936*b*) agrees with it in most respects. The resting cell is cuboidal, non-granular and coarsely reticular with a large, oval, centrally placed nucleus. In the early stages of activity, the cytoplasm is occupied by rounded, basophilic granules of various sizes. These, in developing, change their staining reaction to acidophilic, in the intermediate stages some of the granules being partly basophilic and partly acidophilic. As development proceeds, the now elongated cell is crowded with small eosinophilic granules whose staining reaction is similar to that of the material in the lumen, and the nucleus is usually pushed towards the base. The mature eosinophilic granules are discharged into the lumen and concomitantly the basal protoplasm clears, assuming a fibrillar or net-like structure. The peripheral region, however, remains at first densely staining and is sometimes prolonged into the lumen. Petersen was able to demonstrate granules in the fresh condition which corresponded to those in the fixed cells. Dominici (1913) found that the number of mitochrondria varied with cellular activity and regarded them as taking an indirect part in secretion formation, though not becoming directly transformed into it. Cowdry (1918) described the mitochondria as being very minute and of granular form.

During the third decade there is a gradual, though somewhat irregular increase in the complication of folding of the alveolar epithelium; thereafter, a process of simplification sets in, there being a disappearance of the peninsular extensions of the epithelium so that in the fifth and later decades their numbers are few or they may be altogether non-existent. Because of this, the outline of the alveoli becomes less irregular and there is a concomitant tendency to cystic dilatation. Involutional processes, which seem to commence as early as the fourth decade, are particularly evident in the more peripherally situated alveoli, especially after the age of about 40 years, the epithelium being reduced to a thin, flattened layer of small cuboidal cells, slightly elongated in a tangential direction. These alveoli are frequently dilated (Pl. 4, fig. 21). Actively secreting alveoli seldom disappear entirely from the prostate but, in general, their proportion diminishes with increasing age.

Shedding of the alveolar epithelium is frequently seen in parts of the adult prostate. It occurs probably in two forms: in the first, associated particularly with acute febrile conditions, the whole epithelium may strip off, when histologically, the more or less unchanged columnar cells can be seen in clumps in the lumen. When shedding occurs in the young adult prostate, this is the form in which it is found and it must be considered as resulting, not from a change in the endocrine environment of the epithelial cells, but from the action of toxins. The second form of shedding is associated with hyperplasia of the alveolar epithelium and has not been observed below the age of 35 years. Here, because of a departure in form of the epithelium from that of the normal, androgenically stimulated structure, the whole process must be considered as pathological.

Corpora amylacea. After the age of about 20 years, corpora amylacea are common findings in the prostatic alveoli and have been made the subject of close study by Moore (1936*b*). He regards them as characteristic of involuting and involuted glands. Between the ages of 20 and 40 years, small corpora amylacea are found in about 25 % of prostates; thereafter, they occur with greater frequency. When found in the younger prostates, they are usually associated with some atrophy of the epithelium and are generally from cases of tuberculosis or some other chronic infection. They always differ from those of senile prostates; there are few concentric rings, facets are rare, central pigmentation is unusual and there is no calcification. Central softening is more frequent than in those from senile prostates. The concentric lines typical of corpora amylacea from senile prostates are due to successive deposits and indicate that they take months or years to grow. When concentric lines are found in corpora amylacea from younger prostates they are less distinct, further apart and frequently wavy, while the layers are less compact, all features indicative of softer consistence and less secondary organization and dehydration, reflecting their more recent origin.

Prostatic ducts. The character of the collecting ducts shows considerable variation in adult prostates, and it is by no means easy to draw conclusions as to the association of any particular changes with increase in age. Ducts with a secretory epithelium and others with ordinary cuboidal cells are found, apparently indiscriminately, throughout adult life, although there is no doubt that in old age secretory ducts are the exception rather than the rule. In many cases secretory alveoli are developed in the walls of collecting ducts right up to their openings into the urethra, but occasionally it is difficult to differentiate them from mucosal glands when the latter are particularly richly represented (Pl. 4, fig. 19). Small concretions may be found in the alveoli in the walls of secretory ducts.

Hyperplasia of the duct epithelium seldom disappears entirely from the prostate and, in fact, tends to show an increase in frequency in the fifth and later decades. Pl. 4, fig. 25 shows the hyper-

plastic epithelium of a duct in a 52-year-old prostate; foci of pseudo-acinar formation resembling those of juvenile prostates can be seen in the thickened wall. A gradual falling off in the intensity of androgenic stimulation might be responsible for this, as well as for the less common occurrence of secretory ducts in old age. During the third and first half of the fourth decade, it is almost the rule to find hyperplasia of the ducts in the most antero-lateral extremities of the lateral lobes in the lower half of the prostate. The appearance leads to the conclusion that active proliferation of the glandular system persists longer in this region than elsewhere, with the resultant development of glandular tissue bridging the anterior commissure. In these zones, all the characteristics of proliferation found typically in juvenile prostates are to be seen. Similar hyperplasia and evidence of proliferation is often found in the prespermatic region also. The possible significance of these observations in relation to the development of benign hypertrophy is to be discussed in another publication.

Many of the foregoing points can best be illustrated by reference to the following descriptions of the glandular systems of several prostates of various ages:

H.P. 116—*age* 23 *years.* In the lower part of the prostate anterior extensions of the lateral lobes form a practically complete layer of glandular tissue throughout the anterior commissure. Most of the alveoli are completely differentiated. There are no abnormal epithelial changes. Juvenile hyperplasia is well marked in the collecting ducts, especially those of the most anterior of the lateral lobules.

H.P. 47—*age* 30 *years.* Proliferation of the most anterior of the lateral glands forwards into the anterior commissure is seen; the latter is completely bridged only in the most caudal part of the prostate. These are the only glands which are not fully differentiated. No glands drain into the anterior wall of the urethra. No stratification of the ducts in the prespermatic region is seen; these ducts are secretory right up to their urethral openings.

H.P. 55—*age* 31 *years.* There seems to be a reduction in the proportion of glandular to stromal tissue suggestive of involution. There is some antero-lateral extension, but this does not reach the midline.

In the cranial part most of the alveoli have a normal outline though there is a suggestion of general antero-posterior compression. The epithelium is low and not very active; stratification, up to four layers of cells, is present in parts. There is a little secretion and a few small concretions are present. In the more caudal levels most of the alveoli appear collapsed, being much smaller than normal. Peninsular extensions are small or absent.

Several of the collecting ducts, which open in all the usual situations, are secretory right up to their urethral openings.

H.P. 109—*age* 35 *years.* Most of the alveoli in the cranial part of the prostate present a normal appearance and have a secretory epithelium, though this has fewer peninsular extensions than in a younger adult prostate. There is epithelial shedding in some alveoli. In the remainder of the prostate these changes are more marked and the general appearance suggests that the prostate has experienced a cessation of androgenic stimulation, which has caused some collapse of the alveoli, together with some acute interference which has resulted in extensive ante-mortem shedding of the epithelium which has plugged most of the ducts with masses of cells. In addition, the epithelium of the ducts has been stripped in many places, leaving only a very thin layer of flattened cells with almost invisible nuclei. It is from this basal layer that subsequent regeneration could have taken place.

H.P. 70—*age* 37 *years.* In the cranial levels the glands do not extend anterior to the urethra, but lower down they reach the anterior commissure. In addition, there is a true anterior lobe with ducts draining straight back to the urethra in the caudal half of the prostate.

The cranial alveoli are mostly normal in appearance, though heaping and shedding of the epithelium is seen here and there. At lower levels the peninsular extensions become fewer in number. Secretory epithelium is still the rule but near the periphery there are several alveoli which are elongated tangentially and which have a flattened epithelium. In the most caudal parts, distended cystic alveoli are typical.

In the subcervical region, peri-urethral glands are clearly visible. In general, they are lined by two layers of cells of which the inner layer is of columnar secretory type. Hyperplasia is present here and there.

The epithelium of the ducts is hyperplastic in many places. In some parts the luminal cells are columnar secretory; in others they are more or less flattened cuboidal. Many of the deeper cells are polygonal and must therefore be regarded as metaplastic. Pseudo-acinar formation is to be seen in a few places. The openings of the ducts are situated in the usual locations. In the prespermatic region ducts appear to open right on the summit of the crista.

H.P. 118—*age* 45 *years.* The glandular system has a normal distribution. The anterior commissure is bridged only in the lower part of the prostate. Extensive evidence of involution of the epithelium is seen in all parts. Many of the more peripheral alveoli are dilated and lined by very low epithelium the cells of which have very little cytoplasm (Pl. 4, fig. 21). Many are also filled with concretions.

Hyperplasia of juvenile type is found in many of the ducts in the prespermatic region, as well as in the antero-lateral extensions. In the lower section of the prostate almost all the ducts anterior to the urethra are hyperplastic and show secondary duct formation in their walls.

H.P. 72—*age* 48 *years.* The glandular system has a normal distribution, with a normal proportion of glandular to stromal tissue. Most of the alveoli have a healthy adult appearance, though the epithelium is somewhat irregular and shows shedding in places. They contain secretion and also numerous small corpora amylacea. Some of the peripheral alveoli are dilated, with a flattened epithelium. In the antero-lateral extensions the alveoli have a particularly active appearance. Collecting ducts are secretory right up to their openings into the urethra.

Uterus masculinus. It has already been pointed out in discussing the uterus masculinus of the newborn prostate that this structure assumes a form traceable to one or other of two main types, diverticular or glandular, or to a type intermediate between them. This statement remains true for the adult prostate.

The degree of variation of the uterus masculinus in the adult prostate must be emphasized. Apart from variation of form, there is great variation in extent, in some cases the prespermatic region being almost completely occupied by the utriculus, while in others serial sections are necessary to demonstrate it, so limited is its representation. Nor is there any relationship between the size of the prostate and the extent of the utriculus masculinus; Pl. 4, figs. 24 and 22 show the extensive utriculi of a 22- and a 23-year-old prostate respectively (volumes 20 and 33 c.c.), while Pl. 4, fig. 23 shows the very small utriculus from a 62-year-old prostate which had undergone benign hypertrophy (volume 40 c.c.). (Note that the magnification of figs. 24 and 22 is $\times 11$ while that of fig. 23 is $\times 32$.)

The uterus masculinus opens on the summit of the crista urethralis, usually, but not always, in the median plane and most commonly just proximal to the orifices of the ejaculatory ducts. Occasionally, it opens into one or other of the ejaculatory ducts, and Vintemberger (1926) states that such an arrangement occurs in about 8 % of cases. Sometimes there are multiple openings of the uterus masculinus, while another modification consists in there being a main opening, with some of the diverticula opening independently. In both these latter arrangements it must be assumed that there has been some absorption of Müller's tubercle into the main lumen of the utriculus.

In a few cases, the uterus masculinus may possess two main lumina, which may unite anteriorly to open on to the crista by a common orifice, or may open by two separate mouths. Such arrangements might be regarded as an indication of the bilateral origin of the utriculus from the Müllerian ducts.

The capsule surrounding the utriculus in the adult prostate is generally much less readily made out than in younger prostates. In many cases it is clearly incomplete and the question arises as to whether or no there exist connexions between the utricular glands and the adjacent prostatic collecting ducts. This question becomes important in view of the hypothesis put forward by Cunéo (1936) that the glandular elements in prostatic hypertrophy are derived by the lateral extension of the utricular glands. This matter is discussed in detail in another publication but for the moment it will suffice to state that there can be no doubt that in some prostates connexions do exist between utricular diverticula and adjacent prostatic collecting ducts. In many specimens it is not possible to define the limits of the utricular system by simple histological examination, while it is often impossible to differentiate between glands of utricular origin and those of urogenital sinus origin in the prespermatic region. However, by means of serial sections and projectoscopic tracings from these, it was possible in some cases actually to demonstrate the continuity of glands both with the main lumen of the utriculus and with prostatic collecting ducts. It is important to note that such connexions were demonstrable in prostates of ages well below those in which benign hypertrophy is found, and which showed no evidence of that pathological process (see Pl. 4, fig. 22 and Pl. 3, fig. 17).

Before the age of twenty years, although growth and differentiation of the glands of the uterus masculinus occurs, they seldom show evidence of active secretion. Hyperplasia of the epithelium and foci of pseudo-acinar formation are still common. In the third decade, however, the glands have frequently reached a high degree of complexity and closely resemble the true prostatic glands, having a similar epithelium with numerous peninsular extensions (Pl. 3, fig. 18). Such a condition is usual up to the age of about 45 years, after which, although often present, the glands appear to undergo dedifferentiation and it is relatively seldom that they bear much resemblance to the true prostatic glands. The epithelium in the older specimens is generally composed of small cuboidal cells and some degree of hyperplasia is commonly found. Small corpora amylacea are frequent in the utricular glands. The epithelium of the main lumen generally consists of about two layers of cuboidal cells which, at least in the younger adults, often presents a secretory appearance although in older age it, like the epithelium of the utricular glands, tends to become inactive.

The following descriptions of the utricular systems of several adult prostates of different ages should serve to illustrate and in places extend some of the points made above:

H.P. 44—*age* 22 *years* (Pl. 4, fig. 24). The uterus masculinus consists cranially of a central lumen lying in the anterior part of the crista. More caudally there is a dorsal extension of the utriculus consisting of a main lumen with associated glands and lying between the ejaculatory ducts. Still more caudally the dorsal and ventral components unite to form a single system. Thus, the uterus masculinus is composed of a central lumen which bifurcates above into a ventral and a much shorter dorsal lumen, both extensions, like the main body, being associated with numerous tubular glands. The mouth of the uterus masculinus does not appear in any of the sections; the ejaculatory ducts, however, appear to open into the utricular system. Furthermore, throughout the serial sections of the utriculus it is possible to distinguish between utricular and prostatic glands at all levels, and the evidence does not suggest the extension of

utricular glands into the general body of the prostate in this specimen.

H.P. 38—*age* 23 *years.* At a level below the opening of the uterus masculinus there is a sagitally disposed fibro-muscular bar. At a higher level the uterus masculinus consists of a radiating tree of ducts apparently separated from the prostatic glandular tissue within the crista by a circular band of muscle. At this level there are numerous separate openings of the utricular glands on the crista; it is to be presumed that the main mouth is situated slightly lower down. The main ducts are tubular and are actively proliferating, the epithelium consisting of one or two layers of small cells with oval nuclei. In a few places these cells have differentiated into secretory epithelium. Although, as was mentioned above, the utricular glands appear to be separated from neighbouring glands in the crista by a band of muscle fibres, these other glands so closely resemble the utricular glands that it is impossible definitely to state that they too are not of utricular origin.

At more cranial levels there is a mass of prespermatic glandular tissue unlike properly differentiated prostatic glands, but very similar to the utricular glands. This prespermatic tissue is undoubtedly connected with the outlying prostatic glands, and the fact that the prespermatic tissue at still higher levels consists of fully differentiated prostatic glands very strongly suggests an extensive ramification of Müllerian elements among the true prostatic glands in this prostate.

H.P. 116—*age* 23 *years.* The utricular system consists of a somewhat indefinite T-shaped main lumen with masses of glandular acini on either side, the whole system filling the crista and extending far back between the ejaculatory ducts (Pl. 4, fig. 22). It is impossible, even with the aid of serial sections, to differentiate the limits of the utricular glands and those of the laterally disposed ducts. There is a strong suggestion of extensive lateral proliferation of utricular glands into the anterior parts of the lateral lobes of the prostate. At more cranial levels there are some prespermatic glands and ducts having direct connexions with the utricular glands lower down.

As in some other specimens, the uterus masculinus appears to open not by a single mouth but by means of numerous small ducts; it is to be presumed that part of the main lumen has been absorbed during development into the crista and urogenital sinus. The sections, unfortunately, are not sufficiently clear to enable one to make a definite statement on this point.

H.P. 55—*age* 31 *years.* The uterus masculinus appears to consist of a mass of glandular acini situated in small part between, but mostly in front and on either side of the ejaculatory ducts. The exact limits of the system cannot be defined; it appears to merge with prostatic collecting ducts on either side. The epithelium resembles that of the prostatic alveoli, being folded, with peninsular extensions, and of secretory type. For the most part, however, there seems to be more than one layer of cells.

H.P. 109—*age* 35 *years.* That portion of the utriculus visible in the sections consists of an irregular main body, most of the lumen of which has been occluded, situated moderately far back, together with a series of secretory glands running forward between the ejaculatory ducts, and spreading somewhat laterally in front of them. Some of these appear to open independently on the crista. Although the prostatic collecting ducts appear to be situated a moderate distance laterally from the utricular glands, it is very difficult to be certain of the precise limits of the latter and of their possible connexion with prostatic ducts.

The epithelium of the utricular glands has undergone similar changes to those in the prostatic glands and ducts (see p. 138 where the glandular system is described).

The epithelium of the main body appears to be actively proliferating. It consists of some two layers of small, darkly staining basal cells, and a differentiated luminal layer of tall columnar secretory cells which are so swollen as to have obliterated the lumen in most places. Here and there are to be seen some intraglandular stromal nodules, similar to those seen in the utriculus of the langur (*Presbytis entellus*) subjected to oestrogenic stimulation (Zuckerman & Parkes, 1936*a*). These have a whorl-like appearance, not unlike the nodules found in the urethral stroma in later life, and seem to be surrounded by a definite wall of epithelial cells.

H.P. 70—*age* 37 *years.* The part of the uterus masculinus visible in the sections consists of an irregular main lumen lying between the ejaculatory ducts and giving off a complex series of glandular diverticula anteriorly. Some of these latter pass close to and may in fact be connected with certain of the prespermatic lobules. A few much smaller diverticula also pass a short distance posteriorly.

The epithelium of the main lumen consists basically of some three layers of cuboidal cells with relatively large nuclei. In parts there is hyperplasia and probable metaplasia, the lumen containing a fairly large mass of shed cells. The epithelium of the diverticula is similar to that of the main lumen and again hyperplasia and metaplasia are to be seen in places. Peninsular extensions are present in several of the lumina, but typical columnar secretory cells are to be seen in one or two places only.

H.P. 74—*age* 39 *years.* The uterus masculinus has a coronal main lumen from which a few small diverticula arise anteriorly, while posteriorly a series of glandular alveoli penetrate between the ejaculatory ducts. Anteriorly and laterally there is a well-marked surrounding stromal condensation containing some muscle fibres but posteriorly it becomes indefinite. The epithelium resembles that of the prostatic alveoli except that in the coronal lumen it is composed chiefly of one or two layers of small cuboidal cells; hyperplasia is seen in a few places. Some small corpora amylacea are present.

At a more caudal level the main arrangement is similar to that described above, but here the diverticula extend anterior and lateral to the ejaculatory ducts, although there is no suggestion of involvement with the prostatic ducts. The surrounding capsule is less clearly marked than in more cranial levels.

H.P. 53—*age* 44 *years.* There is a small main lumen, sagittal in disposition, with glandular diverticula extending posteriorly, anteriorly and a short distance laterally in front of the ejaculatory ducts. There is no definite capsule surrounding the organ, but in no place do utricular glands appear to be extending into the region of the prostatic collecting ducts. The utriculus extends cranially between the ejaculatory ducts and is seen as a somewhat angled sagittal slit accompanied by several small diverticula. The epithelium consists of two or three layers of cuboidal cells, with secretory differentiation in parts, especially in the diverticula. There is some shedding of these secretory cells.

H.P. 72—*age* 48 *years.* The utriculus consists of a fairly long sagittal slit from which a few small glandular diverticula spring, chiefly anteriorly. The epithelium is of secretory type, resembling that of the prostatic glands, though there seem to be two or three layers of basal cuboidal cells in most parts. There is a clearly defined stromal condensation surrounding the uterus masculinus and ejaculatory ducts, and containing some muscle fibres, running chiefly in a cranio-caudal direction.

At a level just caudal to the utriculus a median sagittal bar consisting of collagenous and muscular tissue can be seen.

H.P. 23—*age* 49 *years.* The main lumen of the uterus masculinus is an obliquely disposed slit from which there extend several glandular diverticula. On one side these pass in front of the ejaculatory ducts; on the other, the main lumen reaches a comparable position. The glands do not extend very much lateral to the ejaculatory ducts and they do not appear to be associated with the prostatic collecting ducts passing forwards to the crista. Stromal differentiation of the utriculus from the remainder of the prostate is indefinite.

The epithelium of the main lumen is poorly preserved and appears to consist of two or three layers of small cuboidal cells. In several of the diverticula the epithelium consists of columnar secretory cells and is folded, with peninsular extensions.

Ejaculatory ducts. The epithelium of the ejaculatory ducts is probably the least subject to variation in the whole prostate; throughout life it preserves the same characteristics as are found at birth and which have already been described. There is, however, some tendency to irregularity in later life and in some cases it undergoes complex folding with the formation of numerous tongue-like projections, particularly near the terminal parts of the ducts. Throughout their course through the prostate the ejaculatory ducts are enclosed within a fibro-muscular capsule which contains some elastic tissue. At cranial levels this capsule is usually clearly demarcated, and it may continue so distally, when it comes to surround the uterus masculinus lying between the two ejaculatory ducts. In many cases, however, it becomes indefinite in this distal region. The capsule and the stromal tissue enclosed within it are the remains of the mesoderm of the genital cord of the embryo.

V. THE GROWTH CURVE OF THE HUMAN PROSTATE

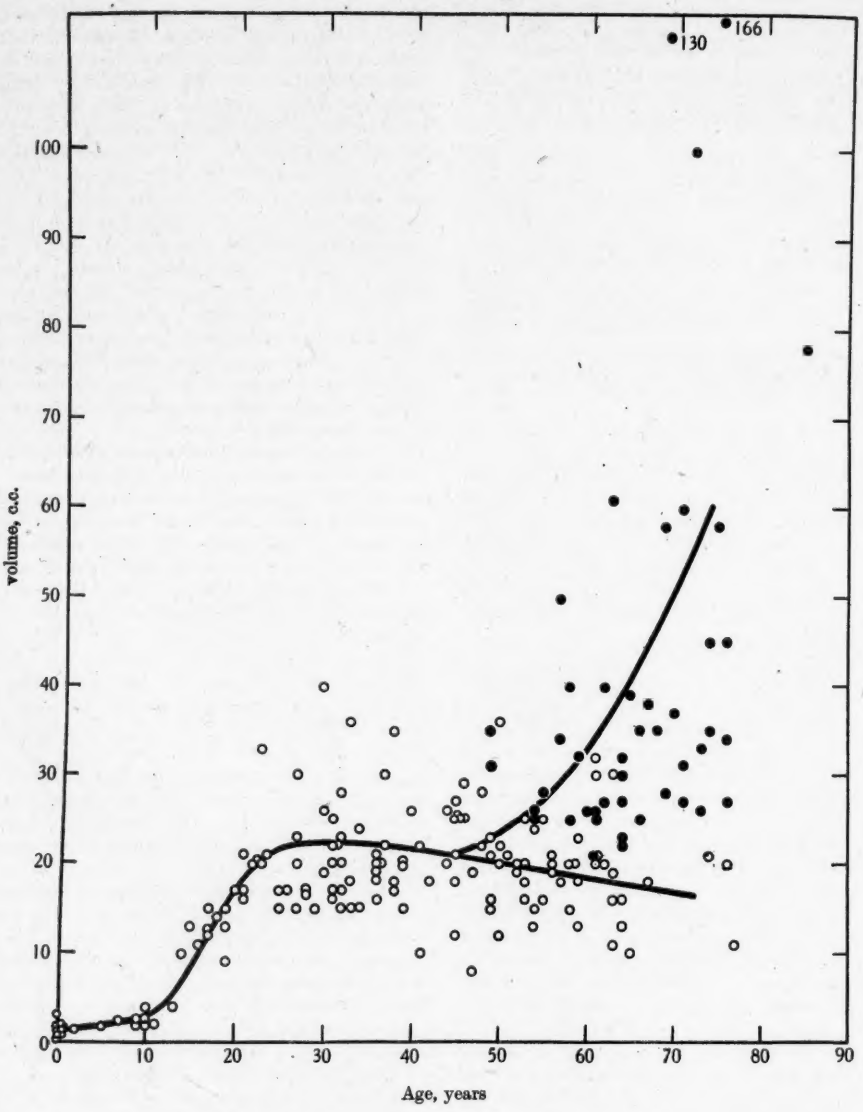
The general tendencies in the growth of the prostate can best be illustrated by means of a scatter diagram correlating age with mass or volume. In the present work, the age-volume relationship has been studied, the method of determining the volume having already been described in the Introduction (p. 130). The practice of fixing the prostates before determining their volumes was adopted from considerations of convenience; it has the objection, however, that it causes shrinkage of the tissue. This is not of very great extent (nor is it very different for the two fixatives used), and since it is greater in the case of glandular than in fibrous prostates (such as are found in old age), it would tend to diminish rather than enhance the growth tendencies shown in the curve. Of the prostates available, there were 192 of which both the age and the volume were known. They have been differentiated into those which show evidence of the pathological changes of benign hypertrophy (even when these were only microscopic) and those which do not (designated 'normal').

Text-fig. 1 is a scatter diagram showing the age-volume relationships of the 192 prostates. Prostates showing hyperplasia are indicated by black circles. Smooth curves have been drawn through the diagram, indicating the mean tendencies of growth in the normal and the hyperplastic prostates.

A further analysis of the growth of the prostate has been made by plotting the mean volumes of successive 5-year age groups. In the age groups 46–50 and on, the mean volumes of those prostates showing hyperplasia and of those that do not have also been plotted separately. The data are to be found in Table 2. Text-fig. 2 shows these data plotted out; hollow circles represent the mean volumes of all prostates in the age group, shaded circles the mean volumes of the normal prostates and black circles those of hyperplastic prostates. The figures refer to the number of specimens in the age group.

It will be seen that the volume of the prostate shows only a very slow increase from the time of birth until puberty is reached. At that time, a rapid increase in size occurs, and is maintained until the third decade is reached, during the latter half of which it levels off and the volume maintains a plateau until the age of about 45 years. Up to this point, the curve is in agreement with that shown by Walker (1922), but in later age groups, two tendencies are manifested, which do not appear in the growth curve of that author. The prostate may either undergo benign hypertrophy, in which case its volume commences to increase at a rapid pace and continues to do so until death, or it may not show this pathological change, in which case it

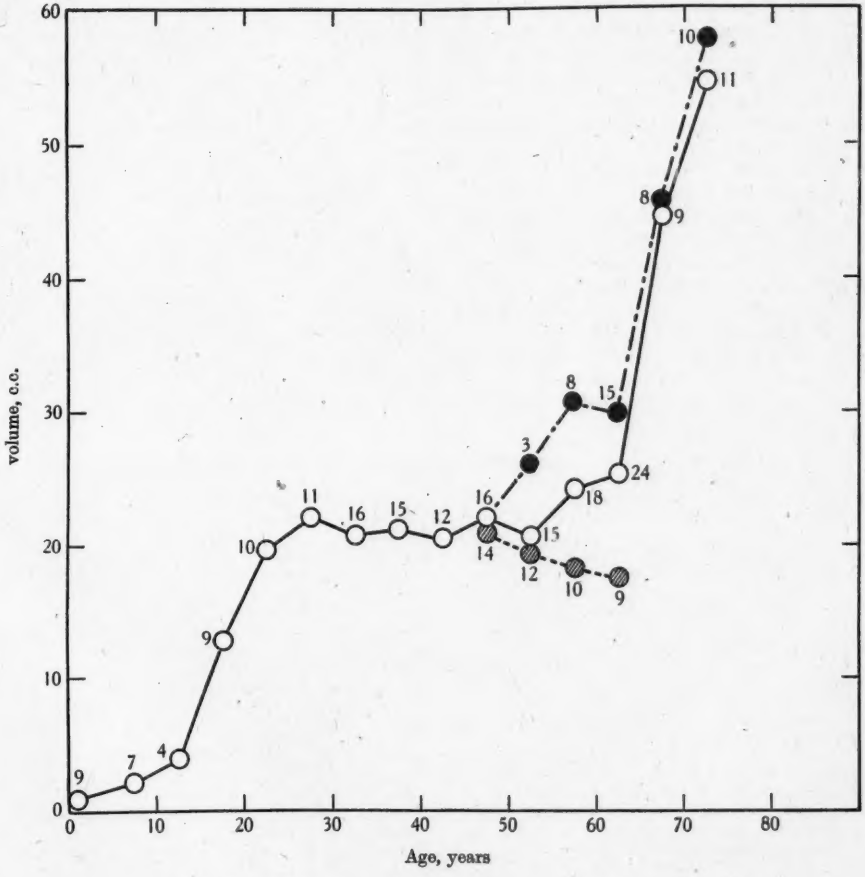
commences to atrophy, its volume progressively decreasing. As has been mentioned already, the atrophy affects chiefly the glandular zones, so that these prostates become relatively more and more fibrous. These alternative growth tendencies in the latter half of life correspond with those found in the prostates of dogs by Zuckerman & McKeown (1938).



Text-fig. 1. Age-volume relationship of the prostate.

Table 2. *Mean volumes of prostates by age groups*

Age group	No. of prostates total	Mean vol. c.c.	No. of prostates normal	Mean vol. c.c.	No. of prostates enlarged	Mean vol. c.c.
0– 2	9	1·4	9	1·4	—	—
5–10	7	2·4	7	2·4	—	—
11–15	4	4·25	4	4·25	—	—
17–20	9	13·4	9	13·4	—	—
21–25	10	20·0	10	20·0	—	—
26–30	11	22·5	11	22·5	—	—
31–35	16	20·1	16	20·1	—	—
36–40	15	21·6	15	21·6	—	—
41–45	12	20·5	12	20·5	—	—
46–50	16	22·3	14	21·1	(2	—)
51–55	15	20·5	12	19·1	3	26·3
56–60	18	24·5	10	18·4	8	30·8
61–65	24	25·5	9	17·8	15	30·1
66–70	9	44·9	(1	18)	8	45·7
71–75	11	54·8	(1	21)	10	58·0



Text-fig. 2. Mean volumes of prostates by age groups.

VI. SUMMARY

1. The embryology of the prostate is briefly reviewed.

2. The prepubertal prostate consists of a radiating series of ducts ending blindly near the periphery and embedded in a fibro-muscular stroma. At birth, hyperplasia and squamous metaplasia are characteristically found in the central parts of the collecting ducts, in the uterus masculinus, and on the crista urethralis. The uterus masculinus is frequently in the form of a distended sac. These features are all the result of stimulation by maternal oestrogens. Evidence of subtotal stimulation by androgens secreted by the foetal testis under the influence of maternal gonadotropic hormone is given by the occasional occurrence of groups of fully differentiated actively secreting alveoli in the prostate at birth.

3. The specific indications of oestrogenic stimulation disappear from the prostate within 6 or 7 weeks of birth. Very little increase in size or differentiation occurs thereafter until about the ninth year. Hyperplasia of the epithelium of the ducts in the central part of the prostate, with pseudo-acinar formation and duct proliferation are characteristic at this stage. By a similar process, the urethral mucosal glands commence development at about this time.

4. At puberty, under the influence of the male hormone whose secretion in effective amounts by the testis now commences, the prostate commences rapid growth and, within 6 months to one year, becomes transformed into the adult type of organ.

5. The arrangement of the glandular tissue in the adult prostate is discussed and contrasted with that found in the foetal prostate. Emphasis is laid on the variability of the alveolar epithelium in adult prostates; part of this is due to the occurrence of a secretory cycle, and part to the occurrence of evolutional and involutional changes. Alveolar epithelial shedding is described as occurring in two forms, related to two main causative factors: toxins and alteration in the endocrine environment.

Hyperplasia of the duct epithelium, so characteristic of the juvenile prostate, is commonly found in the adult organ, and especially in the anterolateral extensions of the lateral lobes; the possible significance of this finding in respect to the development of benign senile enlargement is discussed.

Variability of the uterus masculinus is described, as also is the occurrence of direct communications between utricular and true prostatic glands, from which it may be concluded that some of these latter may be of Müllerian origin in certain prostates.

6. The growth curve of the human prostate is of complex form. Very little increase in size occurs until puberty when there is a rapid enlargement, a high rate of growth being maintained until about the middle of the third decade, after which a plateau is followed until the age of about 45 years. Thereafter, those prostates which do not undergo benign enlargement decrease progressively in size, while those which do recommence a rapid enlargement which persists until death.

I wish to express my indebtedness to Dr A. H. T. Robb-Smith, W. W. Woods and C. F. V. Smout for their kindness in providing the prostates, and to Dr S. Zuckerman for his invaluable guidance during the course of this work. The photomicrographs are the work of Mr W. Chesterman, to whom my sincere thanks are due.

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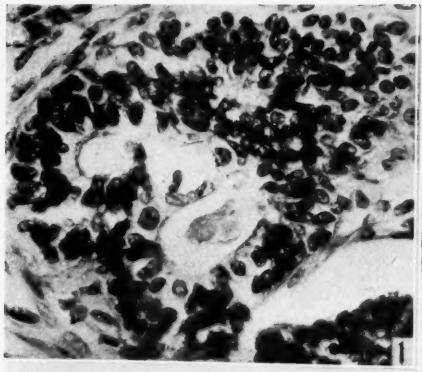
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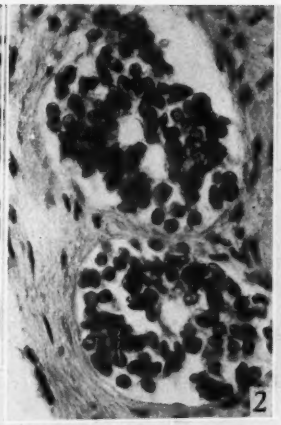
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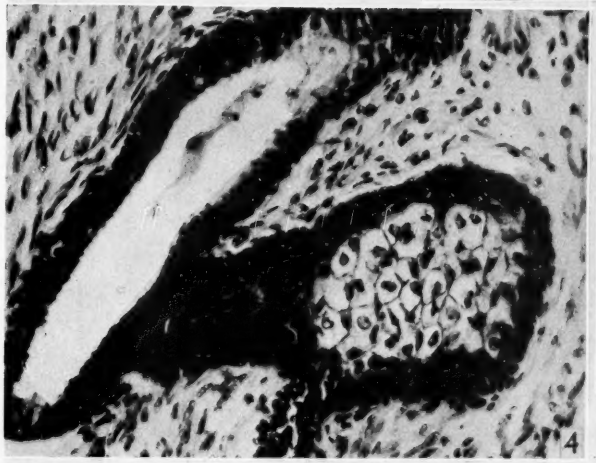
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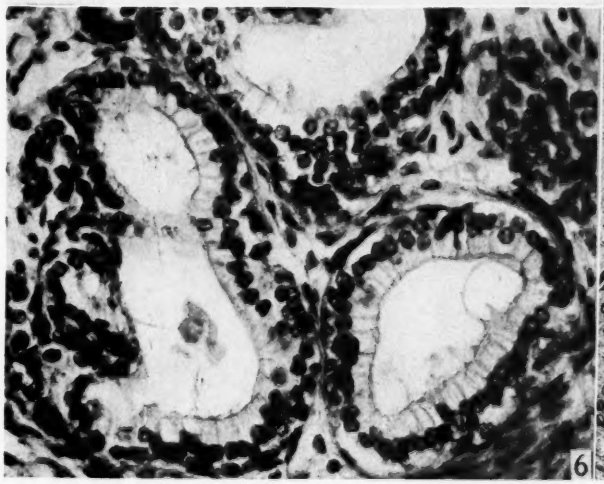
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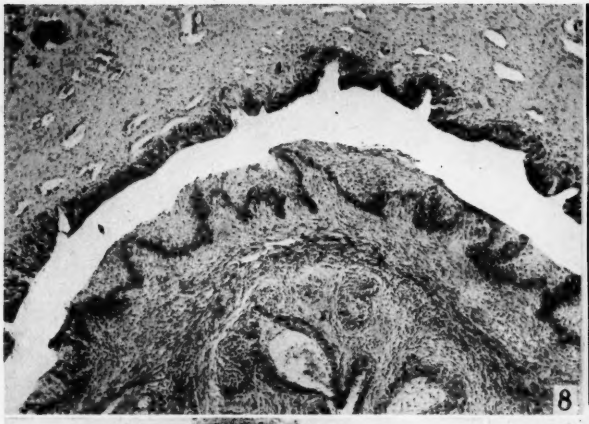
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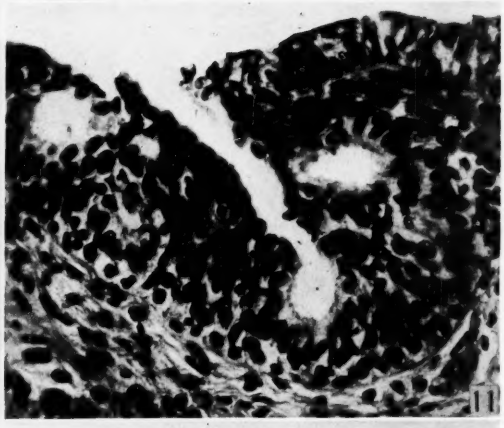
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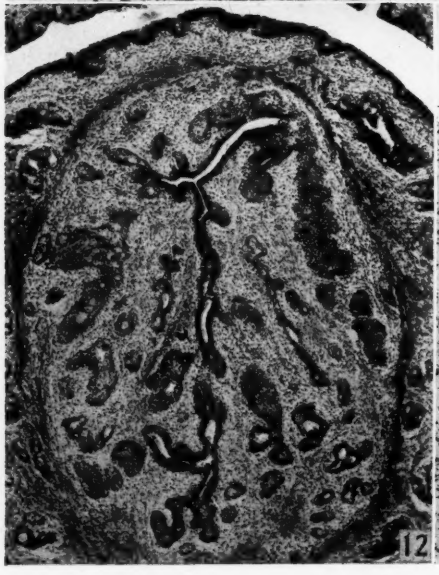
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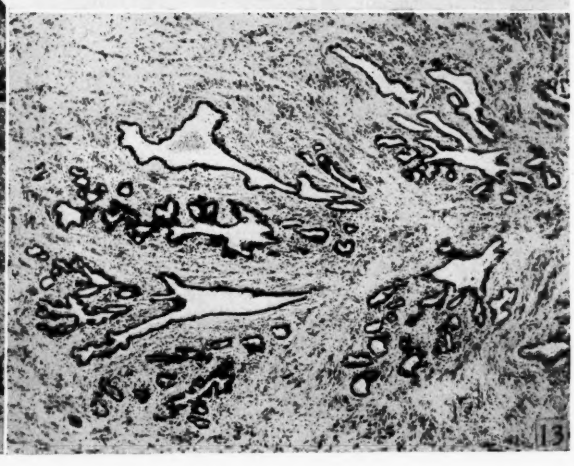
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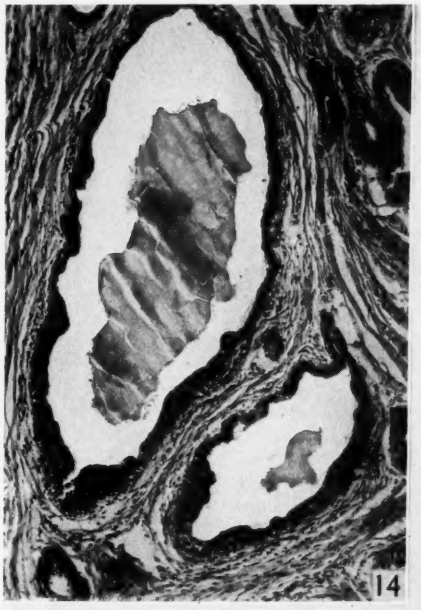
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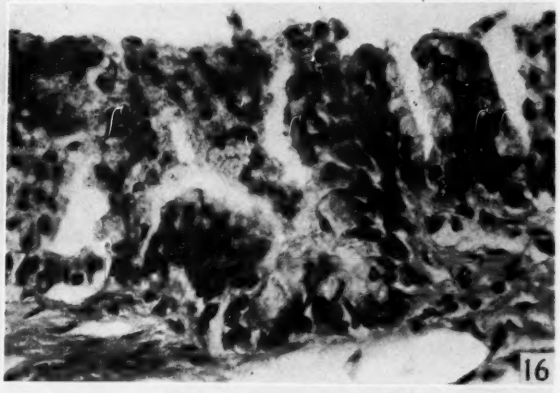
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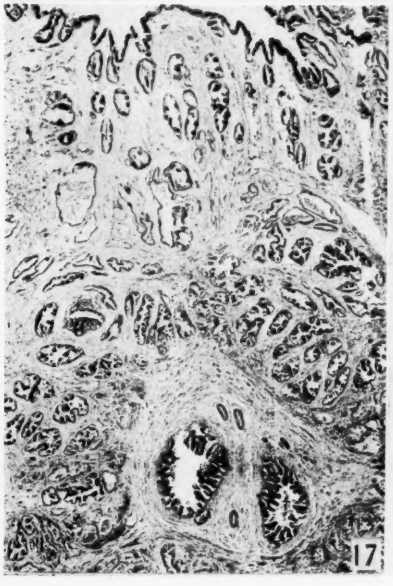
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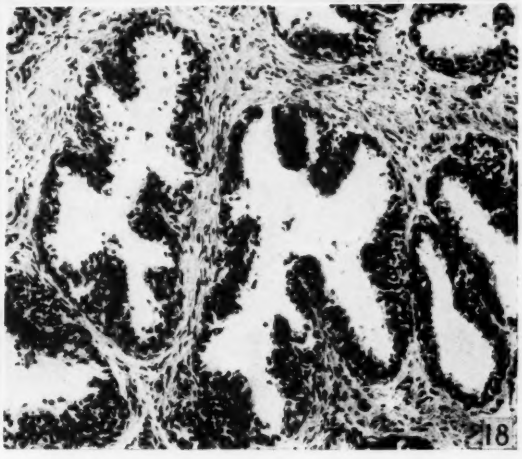
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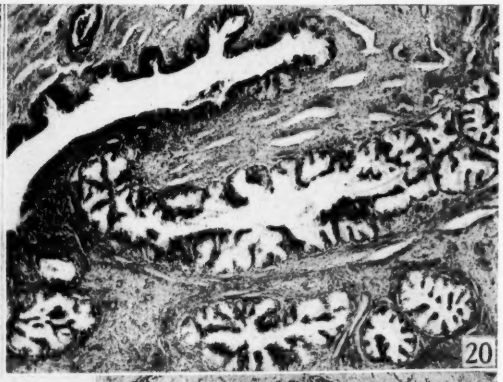
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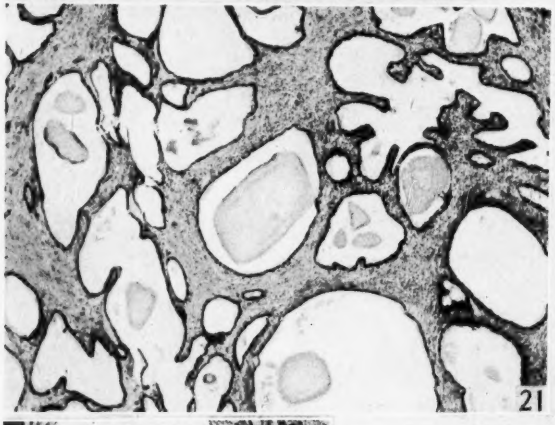
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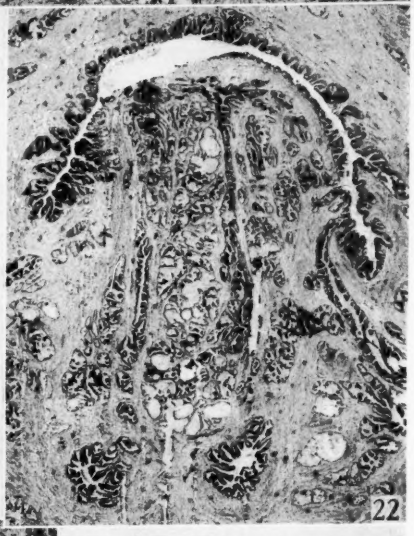
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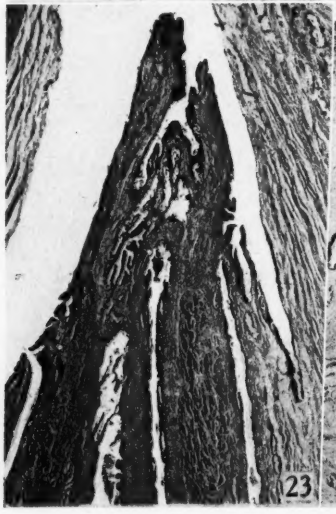
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Pseudo-acinar formation in hyperplastic prostatic ducts at birth; colloid can be seen in one of the lumina. ×370.

Fig. 2. Pseudo-acinar formation in hyperplastic prostatic ducts at birth. ×370.

Fig. 3. Pseudo-acinar formation in hyperplastic prostatic ducts, age 10 years. ×300.

Fig. 4. Hyperplasia and squamous metaplasia in collecting ducts at birth. The ducts are completely or partly blocked. ×300.

Fig. 5. End-buds of the prostatic ducts at birth; some possess a lumen, other do not. ×53.

Fig. 6. End-buds with secretory epithelium at birth; the epithelium consists of tall columnar cells with pale-staining cytoplasm and basal nuclei. Secretory material can be seen adhering to the cells in a few places. ×370.

Fig. 7. The urethra, uterus masculinus and ejaculatory ducts of a new-born prostate seen in transverse section. The utricle is a distended sac with a very thin epithelium. The concentric condensation of connective tissue forming a 'capsule' can be seen. ×22.

PLATE 2

Fig. 8. Transverse section through the verumontanum at birth. The epithelium is hyperplastic and metaplastic, the luminal cells being large and clear, and undergoing keratinization. The anterior wall has not been affected. ×53.

Fig. 9. Prostate from a case of congenital hydronephrosis. The gross specimen has been slit along the anterior commissure and shows the crista urethralis with mucosal valves at the internal meatus.

Fig. 10. Transverse section through a new-born prostate showing a distended uterus masculinus lined by a hyperplastic desquamating epithelium, and containing a large amount of débris in its lumen. The 'capsule' enclosing the uterus masculinus and the ejaculatory ducts can easily be made out.

Fig. 11. Portion of an infolding of the urethral mucosa of a 7-year-old prostate. Four loci of pseudo-acinar formation can be seen. ×370.

Fig. 12. Complex glandular type of uterus masculinus in a 5-year-old prostate. It consists of a T-shaped main lumen with numerous radiating duct-like diverticula, all showing evidence of extensive hyperplasia. There are numerous foci of pseudo-acinar formation. ×35.

Fig. 13. Juvenile end-buds in a 19-year-old prostate; most of the alveoli in this prostate are of normal adult type. ×42.

PLATE 3

Fig. 14. Dilated ducts with hyperplastic epithelium and eosinophilic colloid inclusions in the prespermatic region of a 13-year-old prostate. ×77.

Fig. 15. The prespermatic region of the prostate shown in fig. 14. The uterus masculinus can be seen but is practically indistinguishable from the surrounding ducts, some, at least, of which may in fact be of utricular origin. ×16.

Fig. 16. Hyperplastic epithelium of a prostatic duct in a 15-year-old prostate, showing development of secretory alveoli. ×370.

Fig. 17. The prespermatic region of a 23-year-old prostate. The ejaculatory ducts can be seen posteriorly, and anterior to them is a mass of glandular tissue, partly of utricular and partly of non-utricular origin. By means of serial sections and projectoscopic tracings, interconnexions between these two sets of glands could be traced. ×11.

Fig. 18. Utricular glands in a 41-year-old prostate. Their close resemblance to true prostatic glands is apparent. ×53.

PLATE 4

Fig. 19. A collecting duct with secretory epithelium extending up to the opening into the urethra (which is situated on the left). 49 years old. The urethral mucosal glands are very complex and are practically indistinguishable from the adjacent alveoli of the collecting duct. ×35.

Fig. 20. A collecting duct with secretory epithelium extending up to the opening into the urethra. 15 years old. ×35.

Fig. 21. Dilated peripheral alveoli in a 45-year-old prostate. The epithelium has involuted and several corpora amylacea can be seen, some of them showing concentric rings. ×35.

Fig. 22. The uterus masculinus of a 23-year-old prostate. There is a T-shaped main lumen which is difficult to differentiate from the surrounding, extensively developed, glandular system. It is quite clear that the lateral limits of this latter cannot be defined, and differentiation from the adjacent prostatic collecting ducts is impossible. The ejaculatory ducts can be seen posteriorly. ×11.

Fig. 23. The very small uterus masculinus of a 62-year-old prostate which had undergone benign hypertrophy. Posterior to it are seen the ejaculatory ducts. The manner of opening of the utriculus into the urethra is suggestive of its double origin. ×32.

Fig. 24. The uterus masculinus of a 22-year-old prostate. It can be seen to consist of a main lumen anteriorly, with very extensive glandular diverticula radiating in all directions, but particularly posteriorly. On either side can be seen the ejaculatory ducts. ×11.

Fig. 25. The hyperplastic epithelium of a collecting duct from a 52-year-old prostate. Foci of pseudo-acinar formation can be seen. ×300.

THE EFFECT OF OESTROGENIC STIMULATION ON THE MANGABEY PROSTATE

By F. M. P. ECKSTEIN and S. ZUCKERMAN

A series of experiments was initiated a few years ago to determine whether or not oestrogenic stimulation has any aetiological connexion with benign prostatic enlargement in man. To this end, various species of monkey were investigated in the hopes that amongst them would be found one whose general prostatic structure, and in particular whose utricular structure, was human in character (Zuckerman & Parkes, 1935; Zuckerman, 1940). With possibly one exception, the langur, no such animal was found in the series investigated. As a rule the utricule proved to be a simple urethral diverticulum lined by one or more layers of small cuboidal cells. In response to oestrogenic stimulation this structure underwent extensive stratification and desquamation, but no glandular differentiation and, within the limits of the experiments carried out, there appeared to be no qualitative variation in response correlated with the duration of stimulation. Associated with these prostatic changes, stratification occurred in the crista urethralis, and to a certain extent in the prostatic collecting ducts and in the terminal parts of the ejaculatory ducts. Other changes in the reproductive tract induced by oestrogenic stimulation in the monkeys investigated were:

(i) An increase in the fibro-muscular elements of the seminal vesicles and a concomitant involution of the epithelial tissue.

(ii) Extensive stratification of the whole urethra distal to the prostate.

An opportunity has since presented itself of investigating the response of the uterus masculinus of the Mangabey (*Cercocebus torquatus atys*) to oestrogens. The general structure of the prostate in this species is the same as that in other old world monkeys, with the one exception that there is a slightly more anterior extension of glandular tissue than is normally observed in, for example, the Rhesus monkey (this observation is at present confined to a single immature specimen weighing 7 kg.). The response of the genital tract to oestrogen also appears to be essentially the same as in other catarrhine monkeys. The animal investigated was slightly older than the control, but also sexually immature. It was injected daily, for 42 days, with 100 μg. of oestrone monobenzoate in oil, the total amount administered being 4·2 mg. The utriculus and utricular bed underwent considerable growth, the epithelium of the utricle becoming deeply stratified with extensive desquamation of its superficial layers. As usual the glandular elements of the prostate were unaffected. On the other hand, much more marked hyperplasia and metaplasia had occurred in the prostatic collecting ducts than was observed in other species of monkey. In the upper third of the prostate glandular tissue extended in a well-defined band between the utriculus masculinus on the one hand and the ejaculatory ducts on the other. Hyperplasia and metaplasia had occurred in the epithelium of the collecting ducts of this middle lobe. Unlike the other species of monkey already studied, no changes were seen in the terminal parts of the ejaculatory ducts.

The changes observed in the seminal vesicles, Cowper's glands and the urethra of the Mangabey were the same as those seen in other species of old world monkey, with the main exceptions of a slight degree of the usual type of epithelial changes in the excretory ducts of Cowper's glands, and a certain amount of hyperplasia of the changed epithelium of the seminal vesicles.

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UNUSUALLY LONG PREGNANCY IN A MACAQUE

By F. M. P. ECKSTEIN, *Department of Human Anatomy, Oxford*

The duration of pregnancy in the Rhesus monkey varies, according to Hartman (1932), from 146 to 180 days with an average length of 164 days. The corresponding values for the birth weight in this species are 330 and 600 g. respectively, the average being 435 g. (Hartman & Straus, 1933).

In recent personal communications to Dr S. Zuckerman of this Department, both Dr Carl Hartman and Dr G. Van Wagehen, two American observers who have had more experience in the breeding of these animals than any other workers in this field, give the longest observed pregnancies in their colonies as 180 days; the birth weight in these cases being 520 and 530 g. respectively.

It is of interest to put on record an instance of gestation in the Rhesus monkey which lasted, so far as can be ascertained, 191 days.

The mother was a mature, non-parous macaque whose previous menstrual history had been normal and regular. The animal was first mated early in July 1942 (7 July). Her only one subsequent menstrual period occurred less than a fortnight later (19 July) and was of normal duration. She was delivered of a healthy, though slightly undersized female baby on 8 February 1943, i.e. after a period of 205 days' consecutive amenorrhea. Allowing 14 days for the occurrence of ovulation following upon the last bleeding, it is inferred that the baby's true or 'conception' age was 191 days. It is noteworthy that the weight of the newborn monkey was only 390 g., or about 10% less than the average, and nearly 27% less than in the cases mentioned above in which pregnancy lasted a comparable length of time.

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THE INGUINAL CANAL IN THE FOETUS AND NEW-BORN

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Most anatomists, undoubtedly, are aware that the inguinal canal is formed very early in foetal life, yet it is true that many students graduate from medical schools with the fixed belief that no canal is present at the time of migration of the testis from the abdominal cavity to the scrotum.

There may be several reasons why this belief prevails. The subject may be presented so early in the course that the student does not grasp its significance or fails to appreciate the relative size of the undescended testis and the inguinal canal, or it may be because at least one of the text-books of gross anatomy, widely used in our school, specifically denies the existence of an inguinal canal at the time the testis leaves the abdominal cavity.

Inability to find any published data on the inguinal canal at the time of testicular descent prompted this study. Encouragement to submit our findings for publication has been given by a recent paper by Wyndham (1943) on 'A morphological study of testicular descent'.

W. Felix (1910), in Keibel & Mall, gives a complete and detailed description of the abdominal musculature and the formation of the inguinal canal. He describes the formation of the 'chorda gubernaculi' before there is any differentiation of the abdominal muscles, and says (p. 944) that when the musculature begins to develop 'it must grow around the chorda gubernaculi and thus there is necessarily formed a canal (the inguinal canal) whose contents were there from the beginning'. Other books on developmental Anatomy (Arey, 1940; Jordan & Kindred, 1937) give a similar, but less detailed, description of this process.

Text-books on gross anatomy vary greatly in their presentation of this subject and in the completeness of the details given. Piersol (1930), Cunningham (1937), Grant (1940) and Gray (1942) either describe an inguinal canal or imply that one is present and state that the testis passes obliquely downward and medially through the abdominal wall. Morris (1942) denies the existence of a canal, as is shown by the following statement: 'It is helpful to note that originally this outpocketing extends directly outward (not obliquely), so there is no canal, but one ring lies directly behind the other.'

In the current literature, Watson (1938), and Cherner (1939), in discussing the canal in relation to various types of hernia, confirm the major points previously described by Felix (1910). Howell (1939), and much more extensively Anson and associates (1938, 1941), have written in detail of the development and anatomy of the abdominal wall in relation to the surgery of the inguinal region but without specific mention of the development and the dimensions of the inguinal canal in the foetus.

MATERIAL

The material in this study consisted of twenty-five foetal and infant cadavers, seventeen male and eight female. Clinically, twelve were classed as miscarriage or abortion, three were not classified, nine were classed as 'stillbirth', and one was 24 days old at time of death. The right and left inguinal region and inguinal canal were dissected in each cadaver. As there was no essential difference in the measurements on the two sides only one measurement is included in Table 1. Tabulation is arranged according to foetal length (mm. crown-rump), and not in the order of dissection. All dissections and measurements were made after the material had been 'fixed' or embalmed.

As the margins of the abdominal inguinal ring are difficult to define sharply, measurements were made from the adjacent inferior epigastric artery. By careful dissection and by traction on the contents of the canal, the margins of the subcutaneous ring could always be established. The distance was then taken from the superior lateral margin of this ring to a point on the inferior epigastric artery as noted above.

In the case of the female specimens the margins of the subcutaneous inguinal ring could always be defined by traction on the abdominal part of the round ligaments. Measurements were then taken as in the male specimens.

COMMENTS

Wyndham (1943) reports the inguinal canal well developed in the foetus at 66 mm. (crown-rump) length. In no specimen of 23 cm. or less, in his series, had testicular descent begun, yet it was completed in every specimen, except one, of 24 cm. or over.

From our observations it can be definitely stated that there is an inguinal canal in all foetuses dissected in this study. In our earliest stage it is 4 mm. long; just before testicular descent it mea-

sures 9 mm.; in our older specimens after the testis has descended into the scrotum the canal varies in length from 10 to 15 mm. Similarly, in the female specimens the length of the canal varies from 4 to 16 mm.

After it had been found that a canal was present, even in the youngest foetuses dissected, it seemed of interest to measure the testis in those cases in which it had not yet passed from the abdomen. In these cases the gubernaculum completely filled the canal. It was a firm cylindrical mass and measured from 2 to 3 mm. in diameter. The testis was an elongated, slightly flattened, body from 4 to 7 mm. in length and about 3 mm. in thickness. It would seem, therefore, that this soft mass might easily follow the gubernaculum through the canal without any distortion of either.

In the earlier stages, up to 180 mm., the inguinal canal was almost parallel to the lateral border of the rectus abdominis, but thereafter, with increase in width of the pelvis, it became parallel to the inguinal ligament.

CONCLUSIONS

1. There is a definite inguinal canal in the male foetus before the testis passes through the abdominal wall.
2. The testis at this time is not large enough to disturb the relationship of the inguinal rings.
3. In foetuses of 180 mm. (crown-rump) length or less the inguinal canal lies parallel to the rectus abdominis rather than to the inguinal ligament.
4. The inguinal canal in the female is just as definite and of approximately the same length as in the male foetus of the same age.

Table 1. *All measurements are in mm.*

Sex	Crown-rump length	Between A.S.S. of ilium	Width of rectus at level of A.S.S.	Abdominal ring to rectus	Position of testis A* or S*	Size of testis	Length inguinal canal
M†	130	—	—	—	A	4×3	4
M†	135	—	—	—	A	4×3	4
M	135	—	—	2	A	4×3	5
M	140	27	—	2	A	6×3	5
F	140	—	—	—	—	—	4
M	145	27	13	3	A	6×3	4
F	150	30	13	—	—	—	6
M	158	38	18	—	A	6×3	6
M	170	—	—	—	A	7×3	5
M	177	40	16	2	A	—	8
F	180	38	18	2	—	—	6
F	185	—	—	—	—	—	6
M	250	56	30	—	A	7×3	9
F	250	58	30	6	—	—	9
M	260	58	32	—	A	7×3	9
F	270	60	30	—	—	—	10
M	305	74	32	9	S	—	14
M	310	65	25	8	S	—	10
M	310	75	33	6	S	—	15
M	310	74	34	9	S	—	15
M	310	66	24	4	S	—	11
M	320	79	37	9	S	—	13
F	325	63	41	8	—	—	16
F	340	84	40	5	—	—	13
M‡	355	85	29	—	S	—	11

* A, abdominal; S, scrotal. † No gubernaculum and no evidence of inguinal canal on right side.
‡ 24 days post-natal.

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